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mRNA vaccines: A powerful tool in cancer treatment

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Abstract. As an emerging immunotherapy, cancer vaccine is a method of introducing tumor antigens, genes encoding tumor antigens, immune cells and molecules into the body to activate specific immune responses to kill tumors. In April 2010, the world's first related drug, previz, was approved for marketing in United States. The drug extracts peripheral blood mononuclear cells from patients' autoimmune cells, activates them in vitro and then infuses them back into the treatment of asymptomatic or mildly symptomatic hormone-refractory advanced prostate cancer. mRNA vaccines have attracted much attention because of their high efficacy, specificity, versatility, ability to develop rapidly on a large-scale, low-cost production potential and safety. The mRNA vaccines and nano-agent delivery technology are considered to have great potential in cancer immunotherapy. In recent years, developments in the design and delivery methods of mRNA vaccines have accelerated the development and use of mRNA vaccinations against cancer in clinical settings. This review introduces optimization strategies of mRNA vaccinations against cancer, including coding and non-coding region optimization, and emphasizes mRNA vaccine delivery systems. Multiple nanoparticle vectors have currently been developed to improve mRNA stability and administration efficiency. At the same time, we discuss the applications of cancer therapy with mRNA vaccinations, its limitations and future challenges.

Keywords: Cancer vaccines, mRNA, therapy.

1. Introduction

More and more research is being done on mRNA as a versatile tool for creating novel therapies. Interest in the use of mRNA molecules to transmit antigenic protein instructions for immunization has increased due to the COVID-19 pandemic. mRNA can be produced on a large scale using good manufacturing practices (GMP), and mRNA based human vaccinations are safe [1].

mRNA vaccine mechanism mainly includes the following steps: 1) Delivery and intake: mRNA vaccine after the injections were nearby cells to absorb, especially the dendritic cells (DCs), these cells are efficient antigen-presenting cells, to identify and internalize mRNA. 2) Translation and processing: Internalized mRNA is translated on ribosomes in the cytoplasm to produce antigen proteins. Antigenic proteins subsequently undergo a series of post-translational modifications, including ubiquitination. After proteasomal degradation, TAP, or transport associated antigen processing protein, carries the antigen fragments into the rough endoplasmic reticulum (ER). 3) Antigen presentation: Antigen peptides are transported into the ER lumen by TAP and bind to major histocompatibility complex class I (MHC class I) molecules. Antigenic peptide-bound MHC class I molecules are delivered to the cell surface for presentation [2]. 4) Immune response: activated CD8+ T cells recognize the antigen peptide MHC class I complex displayed on the cell surface, and these T cells release substances like granzyme and perforin

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to cause cancer cells that express the matching antigen peptide to undergo apoptosis [2]. Through the above mechanisms, mRNA vaccines have the ability to elicit an immunological reaction from the body against specific antigens. This process is particularly important for cancer therapy because it can help enhance the body's ability to recognize and eliminate tumor cells.

mRNA vaccines play a crucial role in the field of cancer vaccines. The target of cancer vaccines is tumor-associated antigens (TAs) that trigger immunological reactions in order to eradicate cancer. Antigens specific to and associated with tumors (TSAs and TSAs) are the two categories of TAs. Tumor-associated antigens are non-mutant proteins expressed improperly in cancer cells [3]. Cancer vaccines that target these antigens in clinical studies have not shown great results and may be harmful to the immune system [3]. Neoantigens produced by the genetic instability of cancer cells are among the tumor-specific antigens that are only found in tumor cells and not in healthy cells [4, 5]. Neoantigens have a high level of immunogenicity and are closely associated with the MHC. Due to their exclusive production in cancer cells, they lessen "off-target" damage and activate tumor-specific T cell responses. It has evolved into the primary target of cancer vaccinations in recent years.

In cancer immunotherapy, mRNA-based cancer vaccines have demonstrated a number of benefits: 1) Unlike DNA vaccines, mRNA vaccines do not require nuclear localization to translate antigens. 2) It is possible to effectively and simultaneously administer many antigens. 3) Cross-presenting several epitopes at once in antigen-presenting cells to improve T cell responses and lessen human leukocyte antigen type restriction [2]. 4) It is independent of cell cycle status and can be translated into functional antigen. 5) The production is fast and can be scaled up, which is conducive to industrial production.

Although there are still difficulties in producing high-quality therapeutic mRNAs, coding sequence optimization, capping and tail methods, and the introduction of modified nucleosides like N6-methyladenosine and pseudo uridine have all helped to lessen the inflammatory response. Much emphasis has been paid to the study of mRNA vaccines in cancer therapy, and therapeutic vaccines utilizing mRNA technology for various cancer types are being evaluated in numerous clinical trials.

2. Sequence optimization of mRNA vaccines

A mRNA is made up of a polyadenylate tail, a cancer antigen-encoding open reading frame (ORF) found in mRNA cancer vaccines, a 5'-untranslated region (UTR), and a 3'-untranslated region. It is possible to modify these mRNA components to enhance their immunostimulatory qualities, efficiency and stability in translation. Both optimization of coding regions and non-coding regions are included in the design and optimization techniques.

2.1. Modification with five-prime cap (5' cap)

For mRNA protein synthesis to be effective, the 5'-cap structure is required. The 5'-cap structure controls nuclear export and pre-mRNA splicing, initiates mRNA translation, and protects RNA from exonuclease cleavage. 5' capping can be carried out via an enzyme that caps the vaccinia virus, an anti-inversion cap analogue, or a synthetic cap construct during or after transcription [6].

2.2. Optimization of untranslated regions (UTR)

The mRNA stability, ribosome recognition, and translation are significantly impacted by the5' and3' UTRs that are close to the coding sequence. The efficiency and half-life of mRNA can be greatly increased by optimizing the 5'- and 3'-UTR regions.

Using shorter 5'UTRs that enhance mRNA translation can maximize the efficiency of the 5' UTR sequence. This involves avoiding initiation codons that hinder the translation of open reading frames, as well as preventing the formation of secondary structures that affect ribosome recruitment [6]. The translation and stability of messenger RNA may be improved by the introduction of the 3' UTRs of alpha and beta globin mRNA.

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2.3. Codon optimization of open reading frame (ORF)

Translation efficiency is known to be influenced by codon composition. Replacing rare codons with regular synonymous codons containing a large number of tRNAs can speed up translation and increase yield [7]. Increasing the GC content can also optimize the sequence. The translation efficiency of sequences with rich GC content is 100 times higher than that of sequences with low GC content. Immunogenicity can be decreased, and translation efficiency can be greatly increased by adding chemically modified nucleotides such 5-methylcytidine (m5C) to mRNA [8].

2.4. Polyadenylate tail and mRNA stability

Translation of proteins is facilitated by the binding of messenger RNA through the polyadenylate tail. The translation efficiency is influenced by the length of the polyadenylate tail. By delaying RNA breakdown by RNA exonuclease, the polyadenylate tail increases messenger RNA stability.

There are two different methods for adding a polyadenylate tail to in vitro transcribed (IVT) messenger RNA. Recombinant poly (A) polymerase is one method for extending the IVT mRNA following transcription; Including the poly (A) tail coding region in the DNA template used for IVT mRNA transcription is an alternate strategy. Messenger RNA transcripts created by the enzymatic polyadenylation process have polyadenylate tails of variable length, while the resulting mRNA transcripts have polyadenylate tails of a specific length when DNA is used as a template [6].

3. Delivery of mRNA vaccines

mRNA has potential in cancer therapy but faces multiple biological barriers. The human body's enzymes can readily break down mRNA due to its chemical instability [2]. Due to its high molecular weight and negative charge, mRNA has a tough time passing through cell membranes [9]. Even after entering the cell, mRNA-based drugs cannot escape from the endosome and reach the cytoplasm. Even after chemical modification to enhance stability, only minimal protein expression can be induced by bare mRNA [2]. To increase mRNA stability and transport efficiency, a range of nanoparticle carriers have recently been created.

3.1. Lipid nanoparticles (LNPs)

Lipid nanoparticles, or LNPs, are engineered to shield mRNA against deterioration and transport it effectively into target cells. They are usually composed of four main components: Ionizable lipid, lipid-linked polyethylene glycol (PEG), cholesterol and phospholipids. mRNA can be released from endosomes into the cytoplasm by complexing with lubricant that is soluble in mRNA. PEG, or polyethylene glycol, lengthens the formulation's half-life. The LNP structure is stabilized by cholesterol. Phospholipids support the lipid bilayer structure [10,11].

The structural integrity and phase transition behaviour of LNPs are influenced by phospholipids and cholesterol, and these elements are not anticipated to cause major recognition by the innate immunity or inflammation because they are naturally occurring in mammalian cell membranes [12]. Modularity and adaptability characterize mRNA-LNP vaccines. To achieve various objectives and applications, the composition and ratios of LNPs can be changed. Because mRNA-LNP vaccines can offer higher molecular weights and have a low immunogenicity, large-scale manufacture can be accomplished quickly. Injections into the muscles and skin are the two most commonly utilization of administration in that they have the capacity to offer the maximum degree of immunity and the longest-lasting effects [10]. However, as doses of mRNA-LNP vaccinations increase, so too could their negative effects. The use of mRNA-LNP vaccines has potential risks, including allergic reactions, inflammatory reactions, and long-term immunological changes.

As an advanced mRNA delivery platform, LNPs have a crucial function in safeguarding mRNA and effectively transporting it to desired cells. Nonetheless, more studies are needed to further improve its safety and efficacy.

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3.2. Polymeric nanoparticles (Polymeric NPs)

Polymeric nanoparticles have attracted extensive attention in terms of nucleic acid delivery platforms due to their synthesiability, structural diversity, and stability. Simple repeating units joined by covalent bonds make up these particles. Numerous parameters, such as the concentration and molar ratio of polymer to nucleic acid, as well as the degree of branching and other formulation characteristics, influence the creation of nucleic acid-loaded nanoparticles [1].

The GO-LPEI hydrogel, which is made of cationic low molecular weight polyethylenimine (LPEI) and graphene oxide (GO), is one example of an application. Through π-π stacking and electrostatic interactions, it can encapsulate ovalbumin mRNA (mOVA) and the TLR7/8 agonist rziquinmod (R848) to produce GLP-RO gels. Following a subcutaneous injection, antigen-presenting cells (APCs) in lymph nodes receive GLP-RO nanoparticles that are liberated from the gel, stimulating the immune system and inhibiting metastasis [13]. The second application example relies on charge-altered releasable carriers (CARTs), which are biodegradable amphiphilic poly (carbonate) -B - (α-amino ester) molecules. The cationic amines in CARTs reorganize to neutral amide after entering cells, facilitating the release of mRNA and steady expression. Systemic anticancer immune responses can be triggered against nearby and distant cancers with the local delivery of CARTs containing mRNAs expressing immunomodulatory factors [14]. Shi et al. created a hybrid platform that consisted of a cationic lipid G0-C14-like messenger RNA-delivery nanoparticle and a derivative of ethylene glycol containing methoxy groups A copolymer made of poly(lactic-co-glycolic acid) known as mPEG-PLGA. While the G0-C14 lipid condensed the mRNA, the mPEG-PLGA polymer self-assembled to create nanoparticles. PTEN mRNA is delivered via this hybrid platform. PTEN mRNA stimulates autophagy and damp-associated molecular patterns (DAMP), which in turn triggers anticancer immune responses. When paired with anti-PD-1 antibodies, PTEN mRNA has demonstrated increased therapeutic efficacy [15].

Polymeric nanoparticles offer significant advantages as mRNA delivery systems, including flexibility in their synthesis, structural diversity, and stability. Polymeric nanoparticles are a promising delivery platform for cancer treatment because of these characteristics. Through continuous research and development, polymeric nanoparticles are expected to overcome the current challenges and achieve more effective targeted delivery.

3.3. Nanoparticles composed of peptides (NPs)

Due in large part to their proteolytic properties and ease of production, peptides have long been studied as carriers of nucleic acids, which often allow for effective transfection and good biocompatibility.

For the transport of mRNA, cell-penetrating peptides (CPPs) hold great promise. CPPs, composed of 4–40 amino acids, form nanosized particles with nucleic acids through electrostatic interactions. They can introduce functions like endosomal escape through various amino acid combinations [1]. RNActive is one such example. A bare mRNA and an mRNA complex with cationic protamine make up the RNActive mRNA vaccination platform, which was created by CureVac AG. This adjuvant stimulates the signaling pathways for TLR7 and TLR8. According to multiple pre-clinical animal investigations, RNActive can elicit adequate immune responses [1]. Another example is PepFect14 (PF14). Five of the 21 amino acids in PF14, a cationic amphiphilic CPP, are positive at the N-terminus. In a primary ovarian cancer xenograft model, PF14/mRNA nanoparticles were injected intraperitoneally. Reporter proteins were expressed in a variety of tissues and cells. It performs better than commercial lipid transfectants [16]. In one study, the transfection efficacy of three cations while creating nanoparticles (NPs) with five amphiphilic cell penetrating peptides (CPPs) in cancer cells was compared. These CPPs formed NPs with the mRNA through electrostatic interactions and protected the mRNA from degradation by endonucleases. In cancer cells, cellular uptake and protein expression were only improved by amphipathic CPPs smaller than 200 nm. The creation of complexes with mRNA has been demonstrated to be dependent on the conformational state and physicochemical characteristics of amphiphilic CPPs.

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4. Applications of mRNA vaccines in cancer treatment

Anti-tumor antigen immune responses are stimulated and strengthened by mRNA cancer vaccines. These vaccines bring about an immunological reaction to TAAs or TSAs, increasing the identification and elimination of malignant cells, and provide a long-term therapeutic benefit based on developed immunological memory. Almost every element of the immune response against cancer can be encoded using mRNA. This makes it possible to develop highly complex or personalized cancer treatments.

4.1. In vitro mRNA-based dendritic cell (DC) vaccines

The in vitro mRNA-based DC vaccine is the first clinically demonstrated application of mRNA as a cancer vaccine. Although such vaccines have certain limitations in stimulating T cell responses and clinical efficacy, several studies have shown that they may help to slow or delay disease recurrence, thereby potentially prolonging the overall survival of patients.

The manufacturing process of such vaccines is complex and costly, mainly including the following steps: 1)DC extraction: Blood from the patient is used to extract DC. 2) DC differentiation and maturation: To induce differentiation and maturation, DCs are exposed to cytokines, and at the same time, mRNA encoding antigen is delivered into DCs by electroporation [1]. 3) Vaccine reinfusion: Vaccinations against cancer based on messenger RNA in DC are reinfused into patients by subcutaneous, intralymphatic, or intravenous injection.

For the treatment of pediatric neuroblastoma, adult and pediatric brain cancer, prostate cancer that is resistant to testosterone, melanoma, and cancer of the renal cells, a clinical investigation based on autologous tumor-derived mRNA was conducted [6]. In a clinical study (NCT02808364), investigators used personalized tumor-associated antigen (TAAs) mRNA-treated DC combined with anti-PD-1 medication in patients with glioblastoma. The results showed that TAA-specific T cell responses were generated in the treated patients, and the overall survival rate was good without serious adverse reactions.

In vitro mRNA-based dendritic cell vaccine is a potential cancer treatment method, especially when combined with other immunotherapies such as PD-1/PD-L1 blocking antibodies, showing good safety and preliminary efficacy. However, its complex preparation process and high cost limit its wider application.

4.2. In vivo mRNA vaccinations against cancer

In vivo mRNA vaccination against cancer is currently an active type of mRNA vaccine tested in clinical trials. Compared with mRNA-based dendritic cell (DC) vaccine, it has several advantages, including relatively low cost, convenient production process, and easier scale-up of production. Such vaccines are delivered by delivering mRNA vaccines directly to patients with various cancers, sometimes encapsulated in a delivery platform and sometimes not.

FixVac (BNT111) is a liposomal RNA (RNA-LPx) vaccine against four nonmutant tumor-associated antigens (TAAs) that are prevalent in melanoma. Patients with metastatic melanoma were enrolled in a clinical trial called FixVac (BNT111) to assess the effectiveness of the liposomal ribonucleic acid (RNA-LPX) vaccine. RNA-LPX was administered intravenously either alone or in conjunction with anti-PD-1 antibody. A positive, long-lasting objective response was noted (NCT02410733) [17].

4.3. Personalized mRNA cancer vaccines

TSA is the basis for personalized mRNA cancer vaccines, which are more effective and individualized than vaccines based on TAA. TSA is caused by somatic mutations that happen randomly. Protein sequences that are lacking from ordinary cells are produced by these mutations.

The process of producing personalized vaccines involves taking biopsy samples from a patient's tumor and obtaining genetic information from the tumor by next-generation sequencing. Healthy tissues were aligned to confirm patient-specific somatic mutations, and predictive algorithms were used to screen out neoantigens associated with MHC class I epitopes. Patients received their final customized vaccination after it had undergone additional screening using an in vitro binding test. Encoding many neoantigens with mRNA vaccines is an optimal treatment approach for certain cancer types that include

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dozens of them, independent of HLA type limitation. In their work, Krietier et al. employed a computationally designed mRNA cancer vaccine against many MHC class II neoepitopes, which in pre-clinical testing was able to eradicate tumors entirely.

5. Conclusion

As a powerful and flexible immunotherapy method, mRNA cancer vaccines have shown great potential in the field of cancer treatment. These vaccines can encode and express TAAs, TSAs and related cytokines, thereby inducing immunological reactions. Recent clinical trials have demonstrated the safety and efficacy of mRNA-based cancer immunotherapies. A subset of patients achieved meaningful tumor responses with this therapy. Therapeutic antibodies, immunomodulatory cytokines, antigen receptors, cancer antigens, and other elements of the immune response against cancer can all be encoded by mRNA. Personalized mRNA vaccines, which employ next-generation sequencing (NGS) technology to tailor the production of certain cancer antigens, have created new opportunities for precision cancer therapy. Due to mRNA's temporary nature, its effects are easily managed and help reduce the danger of long-term toxicity and off-target consequences. The development of chemical modification increases the stability and translation efficiency of mRNA and boosts the productivity of encoded proteins. LNPs are immunogenic, which lowers the possibility of adverse consequences, in contrast to viral vectors.

Despite the promise of mRNA cancer therapy, several challenges remain, including the need for further development of methods to improve mRNA stability, the development of safer and more efficient delivery systems, especially for targeted delivery to specific tissues and cell types, and the need for more research to advance the clinical application and development of mRNA vaccines. Especially in combination with other immunotherapies to improve the therapeutic effect. In summary, with the continuous progress of mRNA vaccine technology, the use of mRNA technology in conjunction with distribution mechanism for nanoparticles has demonstrated significant promise for cancer treatment., which not only has made important progress in safety and efficacy, but also provides broad prospects for future personalized treatment. Major advancements in the realm of cancer immunotherapy as well as the effective clinical translation of mRNA cancer vaccines are anticipated.

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