Research Progress of mRNA Vaccines for Cancer Treatment

Yingying Chen
Hubei University of Chinese Medicine, Wuhan, Hubei, China

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Abstract: Cancer has a high mortality rate and a high recurrence and metastasis rate, making it one of the most threatening diseases to humans at present. Traditional cancer treatment methods all have limitations, so it is very necessary to explore new treatment strategies. mRNA vaccines have unique advantages in cancer treatment and have shown excellent therapeutic effects in previous experimental studies. According to the type of RNA used, mRNA vaccines can be divided into four categories. After the cancer mRNA vaccine enters the human body and reaches the target cells, the antigen proteins are translated through ribosomes in the cytoplasm, thereby stimulating the body's anti-cancer immune response. There are many delivery systems for mRNA vaccines, mainly including lipid-based mRNA delivery tools, polymeric nanoparticles, peptide-based nanoparticles, inorganic nanoparticles, and biogenic nanoparticles. Cancer mRNA vaccines can encode tumor-associated antigens (TAAs), tumor-specific antigens (TSAs), CRISPR-Cas9, tumor suppressor factors, cytokines, therapeutic antibodies, etc., each with different advantages and functions. In addition, there are significant differences among mRNA vaccines used for different cancer treatments. mRNA vaccines for cancer treatment still face many pain points, such as the difficulty in determining universal and effective target antigens. In the future, we can continuously optimize cancer mRNA vaccines for these pain points to make them better applied in cancer treatment.

1 INTRODUCTION

Cancer is currently one of the most threatening diseases to humans. Despite ongoing medical advancements, many types of cancer still have extremely low cure rates in advanced stages. For instance, pancreatic cancer, due to its non-specific early symptoms and the current lack of reliable biomarkers for accurate diagnosis, often results in patients being diagnosed at an advanced stage. According to statistics, patients with advanced pancreatic cancer only have a five-year survival rate of the single digits (Rawla et al., 2019). Cancer patients may still experience recurrence after local treatments such as surgery. Additionally, cancer cells can spread through blood circulation, the lymphatic system, and other pathways to other parts of the body. Once the tumor spreads, it significantly increases the difficulty of treatment and causes damage to multiple organs and systems throughout the body.

Traditional cancer treatment methods primarily include surgery, chemotherapy, and radiotherapy, all of which have limitations. Surgery cannot eradicate cancer cells that have already metastasized or spread, has limited applicability for tumors in special locations (such as brain tumors), and carries risks and potential postoperative complications. Chemotherapy, which involves using cytotoxic drugs to kill cancer cells, lacks selectivity, damages normal cells simultaneously, and can cause severe side effects. Furthermore, cancer cells can easily develop drug resistance. Radiotherapy utilizes high-energy rays to destroy cancer cells locally, targeting only the local lesion and causing radiation damage to normal tissues. Therefore, the exploration and research of novel cancer treatment strategies are highly necessary.

In preclinical studies and early clinical trials, mRNA-based cancer vaccine therapy has demonstrated equivalent or better efficacy compared to DNA or peptide platform-delivered cancer vaccines (Huff et al., 2022). mRNA vaccines, as a novel cancer treatment method, are safe and effective. They can be delivered to target areas within the human body through various methods, rapidly translating into antigen proteins upon reaching target cells, stimulating the body's immune response, and effectively killing cancer cells. Moreover, since

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mRNA can encode complete cancer cell antigens, it has the ability to overcome the limitations of human leukocyte antigens (HLA), leading to a wider immune response. Additionally, because mRNA cannot integrate into chromosomes, it does not cause genetic mutations and is relatively safe. Thus, mRNA vaccines represent an excellent cancer treatment method. In the following sections, I will provide a detailed introduction to cancer mRNA vaccines.

2 BACKGROUND INTRODUCTION OF CANCER MRNA VACCINES

2.1 Classification

mRNA vaccines can be divided into four categories: non-replicating mRNA (nrmRNA) vaccines, self-amplifying RNA (saRNA) vaccines, trans-amplifying RNA (taRNA) vaccines, and circular RNA (circRNA) vaccines (Szabó et al., 2022).

NrmRNA only encodes the gene of interest (GOI), along with 5' and 3' untranslated regions. NrmRNA can only replicate the target antigen and cannot self-amplify within cells.

SaRNA vaccines mimic the replication characteristics of alphaviruses. The sequence encoding the GOI and the sequence encoding the RNA polymerase are placed on the same linear RNA. Specifically, saRNA vaccines are modified from alphaviruses. The sequence encoding non-structural proteins is retained, while the region encoding structural proteins is replaced by the sequence encoding the GOI. The non-structural proteins are translated from the sequence encoding non-structural proteins and assembled into an RNA replicase complex. After entering the cell, saRNA can replicate the full-length positive-strand RNA (containing the sequences encoding the GOI and RNA polymerase) and also the RNA encoding only the GOI. Therefore, a small amount of saRNA can self-amplify in the cell to generate a large amount of RNA, thus producing a large amount of target antigen (Beissert et al., 2020).

TaRNA vaccines separate the sequence encoding the trans-replicons (TRs) of the GOI and the sequence encoding the RNA polymerase onto two linear RNAs, avoiding the problems caused by the large and complex molecular sequences in saRNA and greatly improving the translation efficiency.

CircRNA vaccines are more stable than linear RNA because they feature a covalently closed circular structure without the 5' cap and 3' Poly(A) structure.

2.2 Principle of mRNA Vaccines for Cancer Treatment

The mRNA vaccine encoding relevant antigens is delivered into the human body by a selected delivery system. After entering the cell, the target antigen is translated in the cytoplasm through ribosomes and undergoes post-translational modification. The antigen is degraded by the proteasome complex. Some small peptides are transported to the rough endoplasmic reticulum of the cell and are presented by major histocompatibility complex (MHC) class I molecules on the cell surface. After CD8+ T cells recognize the relevant antigens, they are activated and exert a cytotoxic effect, leading to the apoptosis of tumor cells (Kong et al., 2023). At the same time, some antigens are taken up and degraded by cells. The degraded antigens are presented to CD4+ T cells by MHC class II molecules. CD4+ T cells activate B cells to form plasma cells, which produce neutralizing antibodies. Phagocytes are activated through inflammatory factors, thus playing a role in clearing tumor cells (Chaudhary et al., 2021).

3 CLASSIFICATION OF DELIVERY SYSTEMS

3.1 Lipid-Based mRNA Delivery Tools

Lipid-based mRNA delivery tools are currently the most advanced platforms for delivering mRNA in clinical settings. They mainly include lipoplexes and lipid nanoparticles (LNPs), which were previously used to deliver deoxyribonucleic acid (DNA) and small interfering RNA (siRNA), respectively (Estapé Senti et al., 2024).

A lipid-based mRNA delivery system typically consists of the following components: 1. Cationic lipids (lipoplexes) or ionizable lipids; 2. Non-cationic (phospho)lipids; 3. Cholesterol derivatives; 4. Lipids that prevent aggregation (stabilizers, such as polyethylene glycol-lipid conjugates). During the encapsulation process, the aqueous phase containing mRNA and the organic phase containing cationic or ionizable lipids are rapidly mixed to enable the efficient complexation of mRNA with lipid compounds, thus achieving high-efficiency encapsulation of mRNA (Estapé Senti et al., 2024).

Toxicity and liver accumulation are limitations of lipid-based mRNA delivery tools.

3.2 Polymeric Nanoparticles

Currently, poly (β-amino esters) (PBAEs), poly(lactic-co-glycolic acid) (PLGAs), etc., have been studied and can form good delivery systems (Piotrowski-Daspit et al., 2020). For example, Zhang et al. designed a polymeric nanoparticle composed of poly (β-amino esters), polyglutamic acid (PGA), and di-mannose moieties to deliver mRNA encoding transcription factors. The therapeutic efficacy of this polymeric nanoparticle was demonstrated in models of melanoma, glioblastoma, and ovarian cancer (Zhang et al., 2019). In related studies, cationic chemical groups were added to PLGAs, or they were coated with lipids to solve the problem that they cannot complex with nucleic acids under neutral pH conditions (Paunovska et al., 2022; Hasan et al., 2012). Polymeric nanoparticles can be incorporated into lipid delivery systems to deliver mRNA, enhancing their delivery efficiency and stability in serum.

3.3 Peptide-Based Nanoparticles

Currently, peptide-based nanoparticles mainly include cell-penetrating peptides (CPPs) and protamines. The sequences of cell-penetrating peptides are generally very short and have amphipathic regions or cationic regions, enabling them to cross the cell membrane. Amphipathic cell-penetrating peptides are capable of smoothly passing through the cell membrane because of their lipophilic and hydrophilic amino acids. Arginine, histidine, and lysine are found in cationic cell-penetrating peptides. They can interact with and pass through the negatively charged cell membrane because of their positive charges (Shoari et al., 2021). Protamine is an arginine-rich polypeptide that has the ability to condense mRNA into nanoparticles. Protamine has immunogenicity and can stimulate the immune system. For general drug delivery systems, this is a drawback, but for cancer mRNA vaccines, this may be a favorable factor (Kauffman et al., 2016).

Similarly, peptide-based nanoparticles can also be used in combination with other existing delivery systems.

3.4 Inorganic Nanoparticles

Inorganic nanoparticles include gold nanoparticles, mesoporous silica nanoparticles, calcium phosphate nanoparticles, iron oxide nanoparticles, etc. They can be designed into the desired shapes and sizes, and their surfaces are easily chemically modified.

The Ca2+ in calcium phosphate nanoparticles easily binds to negatively charged nucleic acid molecules, and calcium phosphate can easily pass through the lipid bilayer of the cell membrane and be dissolved by the acidic environment of the endosome, making it a very good delivery system (Levingstone et al., 2020). At the same time, since calcium phosphate is an inorganic mineral present in the human body, it has high biocompatibility, biodegradability, and no immunogenicity.

It should be noted that some inorganic nanoparticles cannot be degraded or cleared by the human body, so their toxicological evaluation should be carried out in advance.

3.5 Biogenic Nanoparticles

Biogenic nanoparticles include exosomes, cell membrane nanoparticles, bacterial vesicles, etc. They have good biocompatibility and are non-toxic. Because there are cell receptors on their surfaces, they can avoid being rapidly degraded by the human body, ensuring the effective delivery of mRNA.

4 DIFFERENT MRNA VACCINES FOR CANCER TREATMENT

There is a wide variety of cancer mRNA vaccines, including mRNA encoding tumor-associated antigens (TAAs), mRNA encoding tumor-specific antigens (TSAs), mRNA encoding chimeric antigen receptors (CARs) or T-cell receptors (TCRs), mRNA encoding CRISPR-Cas9, mRNA encoding tumor suppressor factors, mRNA encoding cytokines, and mRNA encoding therapeutic antibodies.

TAAs are overexpressed in tumor cells and have low or no expression in normal tissues. They are non-mutated proteins with poor tumor specificity and immunogenicity. Generally, mammals have a high degree of immune tolerance to a single TAA. Therefore, multiple TAAs are usually selected for cancer treatment with mRNA vaccines (He et al., 2022). However, TAA vaccines also have their drawbacks. For example, TAAs may mutate and

develop vaccine resistance, which limits their application. TSAs are neo-antigens resulting from tumor cell mutations and are not expressed in normal tissues. They have strong tumor specificity and immunogenicity and high affinity for MHC molecules. Since TSAs do not exist in normal cells, they do not cause immune tolerance in the body. They can be well recognized as "non-self" substances by the host immune system, making them very important targets for cancer vaccines with weak "off-target effects". Personalized tumor mRNA vaccines can be designed according to the unique mutation characteristics of tumor cells in cancer patients for personalized treatment. Currently, there are individualized mRNA vaccines encoding multiple TSAs under pre-clinical and clinical research, and good progress has been made. However, this method currently has the disadvantage of high cost.

Both Chimeric Antigen Receptor T-Cell Immunotherapy (CAR-T) and T-cell receptor engineered T cell therapy (TCR-T) belong to adoptive cell transfer therapy (ACT). ACT refers to obtaining T cells from tumor patients or healthy donors, modifying them specifically in vitro to enhance their targeting and killing effects on tumors, and then infusing them into patients for tumor treatment (Rataj et al., 2019). CAR-T uses genetic engineering techniques to equip T cells with chimeric antigen receptors (CARs) that can specifically recognize antigens on the surface of tumor cells in vitro. The gene encoding CAR is mainly introduced into T cells through viral vectors. The modified T cells can recognize tumor cell antigens, does not rely on MHC molecules (Schepisi et al., 2019), activate more efficiently, and thus kill tumor cells more effectively. TCR-T first screens out TCR sequences that can specifically recognize tumor antigens through genetic engineering techniques and then introduces the screened TCR genes into the patient's own T cells, enabling them to express T-cell receptors (TCRs) that can specifically recognize endogenous antigens of tumor cells. The modified T cells are activated by recognizing the antigen peptides of tumor cells presented by human leukocyte antigen (HLA) to better kill tumor cells. Delivering mRNA nanoparticles encoding CARs or TCRs into the human body to reach T cells and genetically reprogramming circulating T cells in the body to directly generate CAR-T cells or TCR-T cells in vivo is a cost-effective treatment method.

CRISPR-Cas9 is a system composed of clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated proteins (Cas). It consists of the Cas9 endonuclease and single-guide RNA (sgRNA) (Li et al., 2018). After the mRNA encoding CRISPR-Cas9 enters the human body and reaches the target cells, the Cas9 endonuclease can be synthesized in the cytoplasm. It forms a Cas9-sgRNA ribonucleoprotein complex (RNPs) with specific sgRNA. The CRISPR-Cas9 system can perform gene editing. First, the sgRNA is used for guiding and positioning. The sgRNA has a specific nucleotide sequence that can base-pair with the target DNA sequence, thus guiding the Cas9 protein to the target site (Liao et al., 2024). Subsequently, the Cas9 protein uses its nuclease activity to cut the DNA double-strand at the specific location. When the DNA double-strand is broken, the cell initiates its own repair mechanism, including non-homologous end-joining (NHEJ) and homologous recombination repair (HR). The NHEJ method is error-prone and may cause the target gene to lose its function. The HR method can accurately repair the broken DNA according to the homologous template. By introducing the required repair template, precise gene editing can be achieved (Liao et al., 2024). Therefore, the mRNA encoding CRISPR-Cas9 can generate RNPs in vivo to complete specific gene editing, thereby achieving the goal of tumor treatment.

The types of mRNA vaccines for cancer treatment are very diverse, and there are significant differences among mRNA vaccines for different cancers. The following briefly introduces two recently studied cancer mRNA vaccines:

4.1 Pancreatic Cancer mRNA Vaccine Based on the S100 Protein Family

Pancreatic cancer is a common digestive tract malignant tumor, known as the "king of cancers". Patients with advanced pancreatic cancer only have a five-year survival rate of the single digits (Rawla et al., 2019), making it one of the malignant tumors with the worst prognosis.

The S100 protein family is one of the ligands of the receptor for advanced glycation end-products (RAGE). It can activate RAGE and downstream signaling pathways, thereby affecting the proliferation, survival, and metastasis of cancer cells (Leclerc and Vetter, 2015). The S100 protein not only plays a role in tumor cells but also affects the tumor microenvironment by regulating the inflammatory response, thus promoting tumor growth and metastasis. Obviously, it is a good starting point for the development of new pancreatic cancer mRNA vaccines. In vaccine development, it is crucial to

select target antigens with high immunogenicity and appropriate receptors. In this study, the vaccine was constructed by linking all selected (CTL, HTL, and B-cell) epitopes of S100A4, S100A6, S100A8, S100A9, and S100A11. A specific delivery system delivers the vaccine to the target cells. And the vaccine binds to toll-like receptors TLR-2 and TLR-4, triggering a series of powerful immune responses (Masum et al., 2024). According to the test results, B cell and T cell expression is increased, dendritic cells (DC) exhibit long-lasting immunity, INF-γ levels rise noticeably, and tumor growth factor-β (TGF-β) expression is suppressed, proving that the vaccine can produce a good immune response. This is a valuable research direction (Masum et al., 2024).

4.2 MRNA Vaccine for Malignant Tumors Caused by HPV Infection

Persistent human papillomavirus (HPV) infection can cause various malignant tumors such as cervical cancer. Therefore, it is very necessary to develop targeted mRNA vaccines.

A related study introduced an mRNA vaccine encapsulated in lipid nanoparticles (LNP) expressing tHA-mE7-mE6. This mRNA vaccine aims to introduce mutations into the E6 and E7 of HPV to eliminate their oncogenicity (Li et al., 2024). tHA is a truncated influenza hemagglutinin protein that can bind to the CD209 receptor on the surface of dendritic cells (DC). tHA is also encoded into the mRNA because the fusion of tHA with mE7-mE6 can help antigen-presenting cells (APC) more effectively take up antigens, thereby better stimulating the immune response. The study shows that in the E6 and E7+ tumor model, the mRNA vaccine expressing tHA-mE7-mE6 has a better therapeutic effect than the mRNA vaccine expressing only mE7-mE6 (Li et al., 2024). After treatment with the mRNA vaccine expressing tHA-mE7-mE6, a strong CD8+ T-cell immune response can be stimulated. At the same time, the tumor infiltration of DC and NK cells increases after treatment, proving that it can induce strong anti-tumor immunity in the peripheral and tumor microenvironments, which is very helpful for the prevention and treatment of E6 and E7+ tumors and has broad development prospects (Li et al., 2024).

5 CHALLENGES AND OUTLOOK

5.1 MRNA Vaccines for Cancer Treatment Still Face Many Pain Points

Firstly, due to the high heterogeneity of tumor cells, it is difficult to determine universal and effective target antigens. TAAs have the problem of self-immune tolerance. Although TSAs have strong specificity, the cost and difficulty of personalized treatment are very high (He et al., 2022).

Besides, after mRNA enters the human body, it may over-activate the innate immune system, inhibit antigen expression, and fail to activate the adaptive immune response well, affecting the killing effect on tumor cells. Moreover, tumor cells can evade the recognition and attack of the body's immune system through immune escape, making it difficult for the vaccine to work effectively.

Last but not least, mRNA molecules are very unstable, requiring a high-performance delivery system. Currently, the delivery efficiency of existing delivery systems is low.

5.2 Suggestions for Future Research on Cancer mRNA Vaccines Based on the above Pain Points

Firstly, we can use precise detection technologies such as gene sequencing and proteomics to better analyze the antigen expression profiles of tumor cells and combine cutting-edge analysis methods to screen the optimal antigen targets (He et al., 2022). Moreover, multiple tumor antigens can be combined to design vaccines.

What’s more, we can combine cancer mRNA vaccines with other treatment methods such as immune checkpoint inhibitors for combined immunotherapy (He et al., 2022).

In the research on delivery systems, new carriers such as polymer nanoparticles and exosomes can be developed to improve the stability of mRNA. The LNP technology can also be optimized to improve the delivery efficiency.

5.3 Outlook for Cancer mRNA Vaccines

mRNA vaccines for cancer treatment are a very valuable research field. In the future, it is very promising to achieve breakthroughs in cancer

treatment, which is worthy of our unremitting efforts and investment.

6 CONCLUSION

There are many studies and clinical trials on cancer mRNA vaccines, which is a very valuable and promising research area, but the research on it is also facing some difficulties, such as difficulty in determining universal and effective target antigens, immune escape of tumor cells, difficulty in ensuring effective delivery of mRNA, and low delivery efficiency of existing delivery systems. In future research, we need to overcome these difficulties in a targeted manner, use precision detection technology to better analyze the antigen expression profile of tumor cells, and screen out the optimal antigen targets, we can combine a variety of tumor antigens to design vaccines, we can develop new delivery vectors and optimize LNP technology to improve delivery efficiency, and continuously optimize and improve cancer mRNA vaccines, so that they can be better used to treat or even cure cancer.

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