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Review

Advances and prospects of mRNA vaccines in cancer immunotherapy

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ABSTRACT

Cancer vaccines, designed to activate the body's own immune system to fight against tumors, are a current trend in cancer treatment and receiving increasing attention. Cancer vaccines mainly include oncolytic virus vaccine, cell vaccine, peptide vaccine and nucleic acid vaccine. Over the course of decades of research, oncolytic virus vaccine T-VEC, cellular vaccine sipuleucel-T, various peptide vaccines, and DNA vaccine against HPV positive cervical cancer have brought encouraging results for cancer therapy, but are losing momentum in development due to their respective shortcomings. In contrast, the advantages of mRNA vaccines such as high safety, ease of production, and unmatched efficacy are on full display. In addition, advances in technology such as pseudouridine modification have cracked down the bottleneck for developing mRNA vaccines including instability, innate immunogenicity, and low efficiency of in vivo delivery. Several cancer mRNA vaccines have achieved promising results in clinical trials, and their usage in conjunction with other immune checkpoint inhibitors (ICIs) has further boosted the efficiency of anti-tumor immune response. We expect a rapid development of mRNA vaccines for cancer immunotherapy in the near future. This review provides a brief overview of the current status of mRNA vaccines, highlights the action mechanism of cancer mRNA vaccines, their recent advances in clinical trials, and prospects for their clinical applications.

1. Introduction

Cancer is currently one of the most challenging problems adversely affecting human health. The unique immunosuppressive state of the tumor microenvironment (TME) attracts much attention in the field of cancer therapy. Cancer immunotherapy aims to boost the patient's anti-tumor immune system, change the TME, promote cancer cell death and ultimately improve the cancer survival rate. Although ICIs have been proved to be effective in treating a variety of cancers, the overall response rate in patients is quite moderate. So, there is an urgent need to

Abbreviation: 3′UTR, 3′ untranslated region; 5′UTR, 5′ untranslated region; ADCC, Antibody-dependent cytotoxicity; APCs, Antigen-presenting cells; ARCA, Anti-retrograde cap analogue; CAR, Chimeric antigen receptor; CAR-T, Chimeric antigen receptor T cell; CDC, Complement-dependent cytotoxicity; CTLA-4, Cytotoxic T-lymphocyte-associated protein 4; DC, Dendritic cell; DMA, Dioleoyl methyl-4-dimethylaminobutyric acid; DOTAP, 1,2-dioleoyl-3-trimethylammonium-propane; eIF4E, Eukaryotic translation initiation factor 4E; ER, Endoplasmic reticulum; fDC, follicular DC; GM-CSF, Granulocyte-macrophage colony-stimulating factor; HCC, Hepatocellular carcinoma; HPLC, High throughput liquid chromatography; HSV-1, Herpes simplex virus type 1; ICIs, Immune checkpoint inhibitors; IFN-γ, Interferon-gamma; IL-2, Interleukin 2; IVT, In vitro transcription; LAMP-1, Lysosomal-associated membrane protein 1; LBCL, Large B-cell lymphoma; LNP, lipid nanoparticles; $M^1\psi$, N1-methylpseudouridine; MHC, Major histocompatibility complex; mo^5U, 5-methoxyuridine; m^5C, 5-methylcytidine; m^6A, N6-methyladenosine; NSCLC, Non-small cell lung cancer; ORF, Open reading frame; OVs, Oncolytic viruses; PAMAM, Polyamide amine; PAMPs, Pathogen associated molecular patterns; PEG, Lipid-anchored polyethylene glycols; PEI, Polyethylenimine; PKR, Protein kinase R; PLGA, Polylactic acid-glycolic acid; PRRs, Pattern recognition receptors; RNAi, RNA interference; SaRNA, Self-amplifying mRNA; s^2U, 2-thiouridine; TAAs, Tumor-associated antigens; TAMs, Tumor-associated macrophages; TaRNA, Trans-amplified RNA vaccine; TCR, T cell receptor; TLR, Toll-like receptor; TMB, Tumor mutational burden; TME, Tumor microenvironment; TNF, Tumor necrosis factor; TSAs, Tumor-specific antigens; T-VEC, Talimogene laherparepvec; UTRs, Untranslated regions; VCE, Vaccinia virus Capping Enzyme.

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enhance the efficacy of current cancer immunotherapies.

Cancer vaccine is a major breakthrough in use of antigens to activate the human immune system against malignant tumors. Cancer vaccines fall into several broad categories, including oncolytic viruses (OVs), cellular vaccine, peptide vaccine, and nucleic acid vaccine.

OVs are natural or genetically engineered viruses that can infect and replicate in cancer cells, thereby directly killing infected cells and inducing systemic anti-tumor immunity [1,2]. Several OVs have entered into clinical trials such as adenovirus, poxviruses, herpes simplex virus type I (HSV-1), and coxsackievirus [3]. Talimogene laherparepvec (T-VEC), the first attenuated HSV-1 vaccine approved by the FDA, encodes granulocyte-macrophage colony-stimulating factor (GM-CSF) and promotes anti-tumor immune response and prolongs the median survival rate of patients with melanoma [2,4–6]. In addition, OVs have a synergistic effect on cancer therapy in combination with chemotherapy, radiotherapy or chimeric antigen receptor T cell (CAR-T) immunotherapy [1,7,8]. It also shows the potential of transforming immunosuppressive “cold” tumors into “hot” tumors [9]. However, OVs are live viruses prone to propagation, which may lead to uncontrollable dose changes and suffering from the host antiviral immune response [10].

Cellular vaccines mainly include dendritic cell (DC) therapy and CAR-T therapy. DCs play an extremely important role in anti-tumor immunity due to their strong capability of antigen recognition, processing, presentation and T cell sensitization. Gene editing techniques such as viral transduction and RNA interference (RNAi), applied to the in vitro production of DCs, address the concern that DCs of ex vivo origin lack true phenotype and function. Sipuleucel-T, which consists of autologous peripheral blood DCs loaded with recombinant protein PA2024, was FDA- approved for the treatment of prostate cancer in 2010 [11]. Currently, DCs are also often loaded with tumor-associated antigens (TAAs), tumor-specific antigens (TSAs), or combined with ICIs to enhance anti-tumor immune response [12,13]. However, at present, most DC vaccines need to isolate DC cells or DC mononuclear progenitor cells from patients, then transfuse them back to patients with tumor antigens (DC/ tumor fusion cells), which is a delicate and time-consuming process. Chimeric antigen receptor (CAR) is a synthetic receptor that enables T cells to recognize TAAs or TSAs in a manner independent of major histocompatibility complex (MHC), which has achieved remarkable success in the treatment of patients with malignant hematological diseases [14]. CAR-T cells targeting the B-cell marker CD19 became the first transgenic cell therapy approved by the FDA [15] , showing significant advantages in the treatment of relapsed/refractory large B-cell lymphoma (LBCL) [16–18]. But CAR-T therapy is far from being utilized in solid tumors due to barriers of autotoxicity, antigen escape, off-target effect, tumor physical barrier, and immunosuppression [19–24].

The peptide vaccine, composed of short peptides that bind directly to MHC-I, represents the most promising traditional vaccine. Polypeptide cancer vaccines utilize tumor relevant antigen peptides to induce tumor-targeting humoral and cellular immune responses. However, peptide-based cancer vaccines are difficult to synthesize in vitro and hard to deliver in vivo, thus short of clinical applications.

To overcome the challenges associated with the in vitro synthesis of macromolecules, such as polypeptides or even full-length proteins, nucleic acid vaccines, including DNA and mRNA vaccines, have been developed. These vaccines encode a variety of target antigens. Through intramuscular or subcutaneous injection and in combination with advanced electroporation transfection technique, DNA vaccine delivers target antigen sequence into cells, which is transcribed and translated successfully in vivo and activates the anti-tumor immune response [25]. While tumor-targeting WT1 DNA vaccine and HPV-positive cervical cancer-targeting VB10.16 DNA vaccine have achieved certain positive results in animal models, DNA vaccines have not been used clinically for cancer treatment due to its high potential risk of integration into the host DNA and relatively low immunogenicity [26].

In contrast, mRNA vaccine only needs to enter into the cytoplasm for translation, which perfectly eliminates the unpredictable risk of nuclear integration [27]. As early as 1978, mRNA vaccine emerged but failed to develop for a long time due to its strong immunogenicity and high instability. Unexpectedly, the outbreak of COVID-19 brought mRNA vaccine back into center stage. Developed by Moderna and Pfizer-BioNTech, mRNA vaccine against COVID-19 achieved unprecedented success, in which uridine (U) of the mRNA encoding coronavirus spike protein was replaced by pseudouridine (ψ) [28]. This chemical modification significantly attenuates the induction of inflammatory cytokines by mRNA vaccine and sustains higher and longer antigen expression. In addition, the advancement of delivery system for mRNA vaccine lays out a solid foundation for its successful application [29]. This review briefly summarizes all known mRNA vaccines, describes in detail the action mechanism of cancer mRNA vaccines and the latest progress and evaluates their prospects for cancer immunotherapy.

2. The concept and advantages of mRNA vaccine

The basic principle of mRNA vaccine is to introduce exogenous mRNA encoding an antigen into somatic cells, which use the mRNA as a template for synthesis of the antigen, thereby activating the human immune system [30,31].

2.1. Preparation of mRNA vaccines

For batch synthesis of mRNA, the most efficient method is IVT, which uses linear DNA as a template to prepare mRNA with the assistance of phage RNA polymerase. The main process steps include transcription of linearized plasmid DNA into mRNA, chemical modification (including 5′-end capping and 3′-polyA tailing), isolation, purification, mRNA delivery system loading, mRNA effectiveness and safety evaluation, mRNA vaccine preparation and filling [32]. In particular, this year's Nobel Prize winners, Katalin Karikó and Drew Weissman, discovered that the pseudouridine (Ψ) modification allows mRNAs to efficiently evade surveillance of innate immune system [33]. Their major discovery in nucleoside base modification has led to the development of an effective mRNA vaccine against COVID-19. (Fig. 1)

2.2. RNA modifications improve mRNA stability

Exogenous mRNAs, upon entering the body, are first recognized by toll-like receptors or cytoplasmic nucleic acid receptors and activate the innate immune response [34]. Therefore, in previous research on mRNA vaccines, a major problem has been the extreme instability of mRNAs synthesized in vitro, leading to their easy inactivation after entering the body. mRNA modification has improved the rate of protein synthesis and the functional half-life of mRNAs.

(1) The 5′ cap is a protective structure that contains an m7G at the 5′ end of the eukaryotic mRNA sequence, which is connected to the first nucleotide with a 5′5′-triphosphate bridge named m7GpppN. It facilitates promoter binding and improves protein translation efficiency [35]. The synthesis of “cap”-like structures and “capsidenzymes” to stabilize mRNAs can be achieved through common capping strategies for IVT mRNA, such as the Vaccinia capping system utilizing Vaccinia virus Capping Enzyme (VCE) and the addition of a cap analog (m7GpppG) at the 5′ end of the mRNA through bacteriophage polymerases [36,37].
(2) Regulatable sequences are added to the 5′UTR and 3′UTR regions, and Kozak sequences are typically added after the 5′UTR sequences to improve translation efficiency [38]. The main function of the 3′UTR is to maintain the stability of mRNA, and mRNA degradation can be inhibited by replacing adenylate-uridylic acid-rich sequences in the 3′UTR region [39].

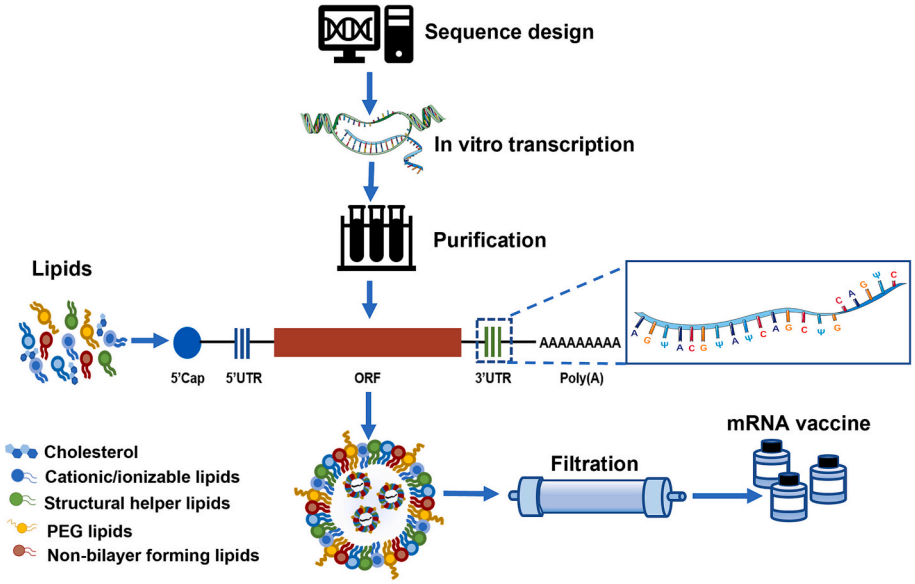

Fig. 1. The preparation process of mRNA vaccine.
The target antigens were screened and designed in silico, transcribed in vitro, and purified by high throughput liquid chromatography (HPLC). Besides encoded peptide or protein. An mRNA vaccine includes the 5′-cap analogue, untranslated regions (UTRs), and poly (A) tail which are designed for enhancing RNA stability and translational efficiency. Importantly, pseudouridine modification reduces the immunogenicity of mRNA vaccine. Purified RNAs are then mixed with lipid to form lipid nanoparticles (LNP). Finally, the filtered mRNA LNP solution is stored in sterile vials.

(3) Adding poly(A) tails regulates mRNA stability, since either shortening or removal of poly(A) tails leads to mRNA degradation [40].
(4) There are many naturally occurring modified nucleotides in the human body, and the host immune system readily recognizes unmodified mRNA vaccines as exogenous molecules, which in turn activate the innate immune response. The use of modifications such as pseudouridine (Ψ), N1-methylpseudouridine ($m^1Ψ$), 5-methoxyuridine (mo^5U), 2-thiouracil (s^2U), 5-methylcytidine (m^5C), and N6-methyladenosine (m^6A) to modify the ribonucleotides of mRNAs reduces the immunogenicity of the mRNAs themselves [33,41]. These modifications concurrently reduce RNA-induced expression of CD80, CD83, CD86 and MHC class II molecules [33]. Of these, pseudouridine (Ψ) is the most abundant modified nucleoside in RNA, and the currently marketed COVID-19 mRNA vaccines, mRNA-1273 (Moderna) and BNT162b2 (Pfizer-BioNTech), both use N1-methylpseudouridine triphosphate (m1ΨTP) instead of uridine triphosphate (UTP).
(5) Codon optimization. Different organisms have different codon-coding preferences for amino acids, and the codons encoding antigens in mRNA vaccines may not be commonly used by the body, so replacing codons with host-preferred ones can help with antigen translation [42]. Additionally, if the ORF region of the mRNA vaccine is rich in GC bases, it is easy to generate secondary structures to hinder protein translation, so reducing the GC content and secondary structures can enhance the antigen production [43,44].

2.3. Advantages of mRNA vaccines

The main advantages of mRNA vaccines are: (1) High efficiency. mRNA can be used as a template for peptide or protein translation. An mRNA vaccine can encode multiple antigens at the same time, which greatly improves its tumor targeting efficacy. (2) Safe and reliable. mRNA will not integrate into the host genome sequences, thus without the risk of insertional mutations [27]. (3) Easy to produce. mRNA can be obtained by in vitro transcription (IVT), which greatly reduces the production time and enables mass production. (4) Flexible platform. Due to heterogeneity, personalized treatment has become the current trend of cancer therapy, and the establishment of mRNA vaccine platform helps shorten the cycle of development of personalized cancer therapy (Table 1).

Table 1
The different types of cancer vaccines.

Vaccine Type	Advantages	Disadvantages
OVs		
	Easy mass production	Live viruses prone to propagation
	High immunogenicity	Host antiviral immune response
		Uncontrollable dose changes
Cell vaccines		
Dendritic cell	Strong capability of antigen, recognition, processing, presentation and T cell sensitization	Expensive and time-consuming
CAR-T	Enables T cells to recognize TAA or TSA in a manner independent of the major histocompatibility complex (MHC)	Unable to be used in solid tumors
		CAR T-cell toxicities
Pepitide vaccine		
	Low toxicity	Difficult to synthesize in vitro
	Little side effects	Difficult to apply to personalized treatment
Nucleic acid vaccines		
DNA vaccines	Delivering several antigen genes in the same construct	High potential risk of insertion into host DNA
	High efficiency	Low immunogenicity
mRNA vaccines	Safe and reliable	Poor stability
	Easy to produce	Certain immunogenicity
	Flexible platform	

3. Classification of mRNA vaccines

There are two main types of mRNA vaccines: non-replicating mRNA vaccine and self-amplifying mRNA vaccine [45].

3.1. Non replicating mRNA vaccine

Non-replicating mRNA vaccines carry full-length mRNA encoding antigen protein transcribed in vitro, which consists of open reading frame (ORF), 5 ′untranslated region (5′UTR), 3′ untranslated region (3′UTR) and poly (A) tail. Elements other than ORF are essential for maintaining the structural stability and the transcriptional efficiency of mRNA. In addition, modifications of these elements extend the half-life of mRNA in vivo and reduce unnecessary immune responses. To boost its immunogenicity and improve the delivery efficiency, this type of mRNA vaccine generally activates the anti-tumor immune response through DCs ex vivo. This strategy has been successful under a variety of experimental conditions and in various clinical settings. For example, to treat triple-negative breast cancer, researchers delivered mRNA encoding the breast cancer-associated antigen MUC1 into DCs in lymph nodes, which activated the proliferation of anti-tumor immune T cells [46]. Another study showed that mRNA vaccine encoding tumor neoantigens in gastric cancer patients successfully induced the activation of $CD4^+$ and $CD8^+$ neoantigen-specific T cells with little side effect. These works demonstrate the safety of the non-replicating mRNA vaccine [47].

3.2. Self-amplifying mRNA vaccine

Compared with non-replicating mRNA vaccine, self-amplifying mRNA (saRNA) vaccine shuns off the shortcomings of the traditional mRNA vaccine and effectively extends the expression of the target antigen [48,49]. The saRNA vaccine consists of three parts: multienzyme replication complexes of viral RNA-dependent RNA polymerase, non-structural proteins, and mRNA encoding target antigens [50]. Virus-like replicon particles synthesize viral structural proteins in vitro and retain certain ability of self-replication. Under the encapsulation by cationic polymers or lipid nanoparticles, the saRNA vaccine is delivered into the cytoplasm through endocytosis of membrane surface receptors [51]. At present, saRNA vaccines encapsulated by bioreducible cationic polymers pABOL and LNP are the most effective in activating humoral and cellular immunity. In addition, based on saRNA vaccine, researchers have developed trans amplified RNA vaccine (taRNA), whose transcription level is 10–100 times higher than that of conventional saRNA vaccine [52]. Since the outbreak of the COVID-19, saRNA vaccine has achieved remarkable reputations in prevention of infectious diseases, but the effect of pathogen associated molecular patterns (PAMPs) may limit its anti-tumor application. Researches on cancer saRNA vaccines are still in the preclinical trial stage (Table 2, NCT01890213,

Table 2
Clinical application of mRNA vaccines in cancer treatment.

Trial ID	Cancer		Vaccine	Route	Phase	Status	Sponsor
NCT01995708	Hematologic malignancies	Multiple Myeloma	CT7, MAGE-A3, and WT1 mRNA-electroporated Langerhans cells (LCs)	i.d.	Phase I	Completed	Memorial Sloan Kettering Cancer
NCT04847050		Lymphoma	mRNA-1273	i.m.	Phase II	Recruiting	National Cancer Institute (NCI)
NCT00834002		Acute Myeloid Leukemia (AML)	Biological: injection of antigen-loaded cultured dendritic cells	i.d.	Phase I	Completed	University Hospital, Antwerp
NCT04163094	Solid tumor	Ovarian Cancer	W_ova1 Vaccine	i.v.	Phase I	Active, not recruiting	University Medical Center Groningen, BioNTech SE
NCT03164772		Non-small Celll Lung Cancer	Biological: BI 1361849	i.v.	Phase I/II	Completed	Ludwig Institute for Cancer Research, Cancer Research Institute, New York City, CureVac AG
NCT00923312			Biological: CV9201	i.d.	Phase I/II	Completed	CureVac AG
NCT00831467		Hormonal Refractory Prostate Cancer	Biological: CV9103	i.d.	Phase I/II	Completed	CureVac AG
NCT02316457		Triple Negative Breast Cancer	Biological: IVAC_W_bre1_uID IVAC_W_bre1_uID/IVAC_M_uID	i.v.	Phase I	Active, not recruiting	BioNTech SE
NCT01526473			Biological: AVX901	i.m.	Phase I	Completed	Duke University
NCT00529984			Biological: AVX701	i.m.	Phase I/II	Completed	Duke University AlphaVax
NCT01890213		Colon Cancer	Biological: AVX701	i.m.	Phase I	Completed	Duke University AlphaVax
NCT04534205		Head and Neck Cancer	Biological: Pembrolizumab Biological: BNT113	i.v.	Phase II	Recruiting	BioNTech SE
NCT01061840		Ewings Sarcoma	Vigil™	i.d.	Phase I	Completed	Gradalis, Inc.
NCT04573140		Adult Glioblastoma	Autologous total tumor mRNA and pp65 full length (fl) lysosomal associated membrane protein (LAMP) mRNA loaded DOTAP liposome	i.v.	Phase I	Recruiting	University of Florida
NCT01456104		Melanoma	Langerhanstype dendritic cells (a.k.a. Langerhans cells or LCs)	i.h.	Phase I	Active, not recruiting	Memorial Sloan Kettering Cancer Center, Rockefeller University
NCT04526899			BNT111 Cemiplimab	i.v.	Phase II	Recruiting	BioNTech SE Regeneron Pharmaceuticals
NCT01066390			TriMix-DC	i.v. / i.d.	Phase I	Completed	Bart Neyns Universitair Ziekenhuis Brussel
NCT05264974			Autologous total tumor mRNA loaded DOTAP liposome vaccine	i.v.	Phase I	Not yet recruiting	University of Florida
NCT00204516			mRNA coding for melanoma associated antigens GM-CSF	i.d.	Phase I/II	Completed	University Hospital Tuebingen
NCT03897881			Biological: mRNA-4157 Biological: Pembrolizumab	i.v.	Phase II	Active, not recruiting	ModernaTX, Inc., Merck Sharp & Dohme LLC
NCT02410733			Biological: Lipo-MERIT	i.v.	Phase I	Active, not recruiting	BioNTech SE

NCT00529984 and NCT01526473).

4. The action mechanism of cancer mRNA vaccine

Each step in the process of developing an mRNA vaccine is critical. As mRNA cancer vaccine evolves toward individualized medicine, the identification of distinct effective antigens determines its efficacy. The delivery vehicles protect mRNA from degradation and promote mRNA loading efficiency and immunogenicity. When mRNA vaccine safely enters cells, it undergoes translation and antigen presentation, ultimately activating the anti-tumor immune response. This section details the action mechanism of cancer mRNA vaccines, focusing on the specific process of immune activation.

4.1. Selection of target antigens

4.1.1. Tumor neoantigen

Genomic instability such as mutation, deletions, inversions, and substitutions of DNA fragments, is one of the key features of tumors. Nonsynonymous mutations in somatic cells produce non-autologous proteins, namely tumor neoantigens (TSAs) [53], which are expressed only in tumor cells, but not in normal cells, and are weakly tolerated [54]. Tumor mutational burden (TMB) determines TSA, that is, the number of mutations of any nature per megabase in tumor tissue [55]. Tumors with TMB >10 somatic mutations/megabase (equivalent to 150 nonsynonymous mutations in an expressed gene) are likely to have correspondingly high numbers of TSA and respond better to mRNA vaccine therapy and ICIs [56].

However, the occurrence of high TMB does not always coincide with better ICI response, but depends on the “qualities” of TSA. These qualities include, (1) Heterogeneity, which refers to the degree of difference compared with wild-type amino acid sequence [57]. (2) Clonal distribution, clonal mutation makes TSA expressed in most tumor cells, while subclonal mutations easily lose expression under the selective pressure of ICIs [58]. (3) Driving mutations, which are more conserved and related to critical functions than non-driving mutations [59]. (4) MHC presentation, the possibility that TSA is properly processed and presented to MHC-I/II molecules with high binding affinity [60]. (5) Affinity of TCR receptor [61].

In summary, it is important to screen and identify effective TSAs. Whole-exome sequencing analysis of cancer tissue samples combined with tumor immune-related data showed that TSA with high TMB was significantly correlated with the increase of tumor infiltrating lymphocytes and the improvement of patient survival rate [62]. Treatment of cancer with mRNA vaccine encoding multiple TSAs finished Phase I/II clinical trial [47]. However, TSA mRNA cancer vaccines still face big challenges such as long-time and high-cost of screening and identification of TSAs, as well as possible changes of the patient's condition during the development of the vaccine.

4.1.2. Tumor associated antigen

Tumor associated antigen (TAA) is an antigen expressed in normal tissues but overexpressed in tumor tissues. It has the characteristics of weak tumor specificity, strong central immune tolerance and weak immunogenicity [57]. Although TAA has many drawbacks, it has broad spectrum and may be a better choice for patients with low TMB. Nowadays, it has become a trend to use multiple TAAs combinations to develop mRNA vaccines. In 2009, Weide et al. conducted a phase I/II clinical study in 21 patients with melanoma using mRNA vaccine encoding six TAAs of melanoma with GM-GSF as an adjuvant. The results showed that the vaccine significantly reduced the number of immunosuppressive cells ($Foxp3^+/CD4^+$T cells) without adverse effects [63]. In 2020, Lipo-MERIT, an mRNA vaccine encoding four TAAs targeting melanoma in combination with PD-L1, successfully inducing interferon-gamma (IFN-γ) secretion and promoting antigen-specific T cell recruitment (NCT02410733) [64]. Moreover, several vaccines encoding TAA and TSA have entered into clinical trials [65,66].

4.1.3. Immunomodulators and tumor suppressor genes

The TME is an attractive target for cancer immunotherapy. Modulation of immunomodulators and tumor suppressor genes may reactivate anti-tumor immune responses [67]. The IL-23/IL-36γ/OX40L triple mRNA vaccine triggered recruitment of large amounts of immune cells into the tumor, and the mRNA vaccine encoding T-cell co-stimulator OX40L significantly improved the response rate of untreated distal tumors [68]. Furthermore, the combined application of this vaccine with ICI improved efficacy in an ICI resistance model [68]. In another study, mRNA vaccine encoding the tumor chemokine CCL2/CCL5 was delivered into the tumor via LNPs, which significantly induced the polarization of macrophages toward anti-tumor M1 phenotype in tumor-associated macrophages (TAMs), and in conjunction with anti-PD-L1 remarkably prolonged the survival time of tumor-bearing mice [69].

mRNA vaccine encoding tumor suppressor genes can treat cancers with mutant or defective tumor suppressor genes. Research showed that mRNA vaccine encoding tumor suppressor gene phosphatase and tensin homolog (PTEN) induced autophagy and apoptosis of PTEN deficient or mutant prostate cancer cells through phosphatidylinositol 3-kinase-AKT pathway, and significantly inhibited tumor growth [70,71]. In addition, an mRNA vaccine encoding the tumor suppressor gene p53 hindered the growth of p53-deficient hepatocellular carcinomas (HCC) and non-small cell lung cancers (NSCLC) by inducing cell cycle arrest and apoptosis, and significantly increased the sensitivity of tumor cells to Everolimus [72].

4.2. Delivery system (Fig. 2)

After the mRNA vaccine arrives near the host cell, the negatively charged mRNA is delivered by interacting with cationic lipids such as 1,2-dioleoxy3-trimethylpropane ammonium chloride (DOTAP) and dioleoyl methyl-4-dimethylaminobutyric acid (DMA) [73]. Lipid nanoparticles are coupled with cell membrane targeted ligands and endocytosis into cells. The endogenous anionic lipids then destroy the delivery complex structure by binding to cationic lipids and release mRNA into the cytoplasm [74]. How to safely deliver mRNA into the cytoplasm for translation is a vital step for successful cancer therapy with mRNA vaccine. The major current delivery methods for mRNA vaccines include LNPs delivery, polymer delivery, peptide delivery, dendritic cell delivery and naked mRNA delivery [75].

4.2.1. LNPs delivery

LNPs are the most widely used mRNA delivery vehicles. They typically consist of ionizable lipids, phospholipids, sterols, and lipid-anchored polyethylene glycols (PEG), among which ionizable lipids are most important for mRNA expression. Collectively, these components facilitate the entry of mRNA into the cell membrane, and stabilize the structure of mRNA [76]. LNPs are biocompatible with cell membrane and protect mRNA from degradation by RNases [77,78]. Efficient mRNA delivery is a major obstacle to improving antitumor efficacy [79]. Lipid-polyethylene glycol (lipid-PEG) coated adjuvant pulsed mRNA vaccine nanoparticles, a formulation that stabilizes the encapsulated adjuvant, significantly improved the transfection efficiency of mRNA [80]. In 2020, Ugur Sahin et al. reported elevated efficacy of RNA-LPX vaccine in melanoma patients treated with ICIs [81]. In addition, subcutaneously injected LNPs-encapsulated mRNA vaccine was captured and transported into lymph nodes by antigen-presenting cells (APCs) at the injection site. Then, the delivered mRNA is translated and presented to $CD4^+$T cells or $CD8^+$T cells via MHC-I/II molecules, activating cellular or humoral immunity [29].

4.2.2. Polymer delivery

The mRNA delivered via polymer has the advantage of being less susceptible to degradation and highly efficient in protein expression.

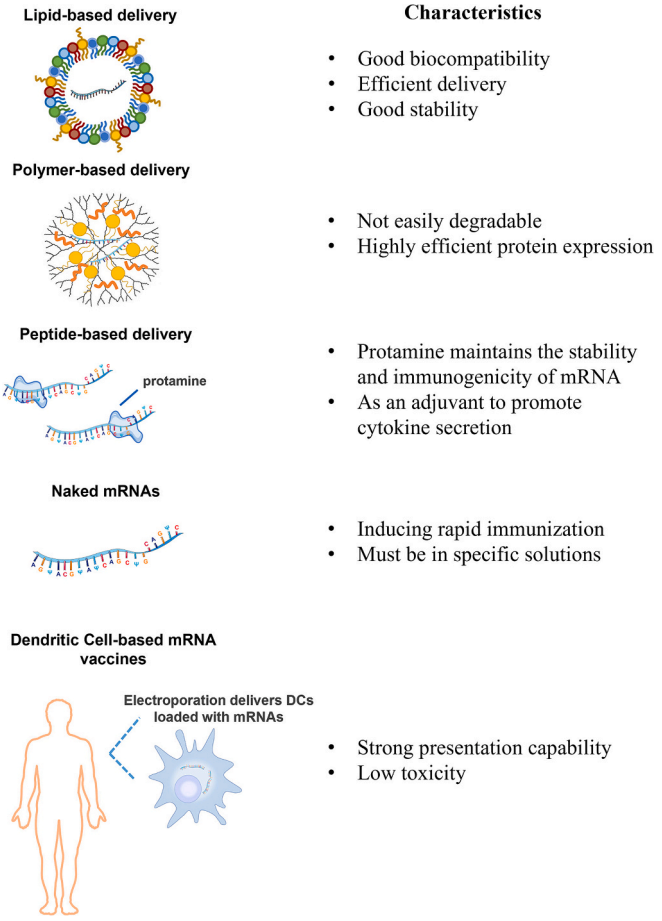

Fig. 2. Delivery systems for cancer mRNA vaccines.
The major delivery methods for cancer mRNA vaccines include: lipid delivery, polymer delivery, peptide delivery, dendritic cell delivery, and naked mRNA.

Two types of polymers, cationic polymer and anionic polymer are used for mRNA delivery. Cationic polymers include polyethyleneimine (PEI), polyamide amine (PAMAM) dendritic macromolecules and polysaccharide [82]. As mRNA is negatively charged, cationic polymers are commonly used for mRNA vaccine delivery. The results showed that PEI protected saRNA from RNase [83]. In addition, a polymer consisting of PAMAM dendrimer, ceramide PEG and polylactic acid-glycolic acid (PLGA) encapsulated mRNA encoding PTEN inhibited tumor growth. PLGA is an anionic polymer that does not deliver mRNA alone. However, the addition of cationic lipids into the PLGA complex significantly improved the delivery efficiency [84]. Nevertheless, due to the large size and limited biodegradation rate of most polymers, this delivery method warrants further improvements [85].

4.2.3. Peptide delivery

As the polypeptides are typically positively charged, the negatively charged mRNA can be delivered by cationic polypeptides through electrostatic interaction [86]. Protamine is one of the cationic peptides that deliver mRNA. On one hand, it protects mRNA from degradation by RNase [87]. On the other hand, it serves a good adjuvant for the secretion of TNF-α and IFN-α by mRNA-activated DCs. CureVac's RNActive technology uses protamine as mRNA carrier. Under various temperature conditions, protamine preserves the immunogenicity of mRNA without affecting the translation of mRNA-encoded antigens [88]. Additionally, in a mouse model of glioblastoma, compared with naked nucleic acid adjuvants, protamine displays much better antitumor effect [89].

4.2.4. Dendritic cell delivery

DCs are important antigen-presenting cells with powerful capacity for uptake, processing, and transporting biomaterials, making them ideal vaccine vehicles [90,91]. The most common technique for delivering antigen-encoding mRNA into dendritic cells is electroporation. This delivery strategy is now being extensively investigated for mRNA cancer vaccines [92]. It has been shown that transfection of DCs by electroporation of mRNA encoding the immune adjuvant TriMix and tumor-associated antigens, followed by infusion of these cells back into melanoma patients, prolonged their progression-free survival [93]. In addition, the delivery of mRNA encoding the tumor antigen MUC1 into DCs activated anti-tumor-specific-T cells, which when in combination with cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) antibodies, significantly improved the treatment efficacy in triple-negative breast cancer [46].

4.2.5. No carrier

Direct injection of naked mRNA often induces human immune response and leads to the aggregation of serum proteins and the degradation of mRNA. Therefore, in the absence of a carrier, naked mRNA needs to be dissolved in specific solutions and transmitted into cells by directly penetrating or destroying the cell membrane. The commonly used solutions for dissolving naked mRNA are Green's solution and Green's lactate solution, in which calcium is beneficial for destroying the cell membrane and thus promoting the uptake of mRNA [94]. Research demonstrated that after the injection of a naked mRNA vaccine encoding TLR7/8 into the lymph nodes of C57BL/6 mice, the expressed TLR7/8 promoted the activation of immune cells, leading to cytokine production, and innate and adaptive immune responses [95]. The advantage of naked mRNA vaccine is that the translation process for the injected mRNA will be initiated immediately after it reaches the cytoplasm, thus achieving rapid immunization.

4.3. Translation

Once mRNA enters the cytoplasm, its efficient and stable translation is a critical step for the success of mRNA vaccine. The 5'cap recruits the eukaryotic translation initiation factor 4E (eIF4E) to facilitate ribosome recognition and translation initiation [96]. During IVT, capping was achieved by adding dinucleotides with 5′-5′ triphosphate linkages, but the efficiency was rather low. The conventional caps (m7GpppG) compete with GTP and are incorporated into mRNA in both directions, resulting in two RNA populations. Half of the mRNA is improperly capped and cannot be recognized by the translation initiation factor, leading to extremely low translation efficiency [97]. The introduction of anti-retrograde cap analogue (ARCA) solved the problem by replacing the 2′ or 3′ hydroxyl groups with methoxy groups $m^{7,2'O}$GpppG and $m^{7,3'O}$GpppG respectively [98]. In addition, following the principle of codon degeneracy, the translation efficiency of mRNA is further improved by replacing rare codons with synonymous common codons. Furthermore, the stability and translation efficiency of mRNA vaccines were also improved by extending the poly (A) tail [99,100].

4.4. Antigen processing and presentation

After the mRNA is translated in the cytoplasm, the synthesized protein is hydrolyzed by protease into peptides which are transported into the endoplasmic reticulum (ER), loaded on MHC-I molecules, delivered to the surface of APC membrane through secretory pathway, and presented to $CD8^+$T cells [101]. As intracellular translated proteins are endogenous antigens which cannot effectively enter the MHC-II pathway. To overcome this obstacle, trafficking signals residing in the MHC-II processing region from endosomal or lysosomal proteins such as lysosomal-associated membrane protein 1 (LAMP-1), chaperones calreticulin, MHC-II associated invariance chains, and HIV TAT protein are fused to the encoded antigens [102]. In addition, the secretory signal

and transmembrane structural domain of MHC-I molecules are incorporated at the N and C terminals of mRNA, respectively, so that the secreted antigen can be re-internalized into the MHC-II delivery pathway, which in turn improves the response of $CD4^+$ and $CD8^+$ T cells.

4.5. Immune activation (Fig. 3)

Appropriate immune response is crucial for the success of mRNA vaccines. Insufficient immune stimulation can lead to the loss of T cells and tumor tolerance. Peptide or protein vaccines exhibits low immunogenicity and require adjuvants such as aluminum salt (Alum), monophosphate lipid A, or Toll-like receptor (TLR) agonists to provide immune stimulation. In contrast, mRNAs can be recognized by pattern recognition receptors (PRRs), which themselves possess immunostimulatory functions. In conjunction with TLR3, TLR7, or TLR8, mRNA vaccine triggers the production of IFN-γ in vivo. IFN-γ is a major regulator of inflammatory factors such as Th cytokines, chemokines, and their receptors, thereby regulating the immune environment [54].

The systemic immune response induced by mRNA vaccines raises concerns in the field. Currently, IFN-γ mediated innate immune response can be reduced by IVT or purifying mRNA with HPLC, as well as through codon optimization and nucleotide modification [34,103]. Alexandra Flemming et al. also found that the use of mRNA containing M1ψ could disrupt the binding of mRNA to TLR7 and significantly reduce the inflammatory response [104]. Moreover, activation of dsRNA-recognized protein kinase R (PKR) should be avoided, as triggering this signal pathway affects the translation of mRNA [32]. The addition of naturally occurring modified nucleosides, such as 2-thiouridine (s^2U), N6-methyladenosine (m^6A), 5-methylcytidine (m^5C), or pseudouridine (Ψ) during transcription in vitro hinders dsRNA formation and thus inhibits PKR activation [41].

Anti-tumor T cells are the main effector cells that mediate the therapeutic effect of mRNA vaccines. In the environment of polarized cytokines created by mature DC, T cells migrate to and infiltrate into tumor tissue, and actively induce and recognize costimulatory signals on the surface of antigen-presenting DC [105,106]. Interaction between MHC-antigen peptide-T Cell receptor complex and homologous receptor-ligand pair (CD80/86; CD70/27) induces DC to secrete cytokines and activates T cells [107]. Activated $CD8^+$T cells secrete IFN-γ, tumor necrosis factor (TNF), granzyme, and perforin, which act on target cells [108]. Interleukin 2 (IL-2) secreted by $CD4^+$T cells promotes the proliferation of $CD8^+$T cells and further assists the direct killing effect of $CD8^+$T cells [109]. Meanwhile, activated T cells and follicular DCs (fDCs) jointly promote the production of plasma cells and memory B cells to kill tumor cells through antibody-dependent cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) [110,111]. In addition, mRNA vaccines encoding immune-modulating molecules are usually injected together with mRNA vaccines encoding multiple tumor antigens, which induce DC maturation and antigen-specific T cell initiation in situ [112].

5. Improvement of mRNA vaccine

The mRNA synthesized through IVT has two major drawbacks: (1) The mRNA has certain immunogenicity; (2) Poor stability. Uracil-rich mRNA sequences are important for activation of TLR and induction of TLR-mediated innate immune response. Earlier in 2005, Drs Katalin Kariko and Drew Weissman in the University of Pennsylvania found that uridine in the mRNA sequence was a key factor in mediating the unwanted immune response. When uridine (U) modified into pseudouridine (Ψ), the mRNA escaped the surveillance of the immune system [28]. This breakthrough solved a key problem for mRNA vaccines. The

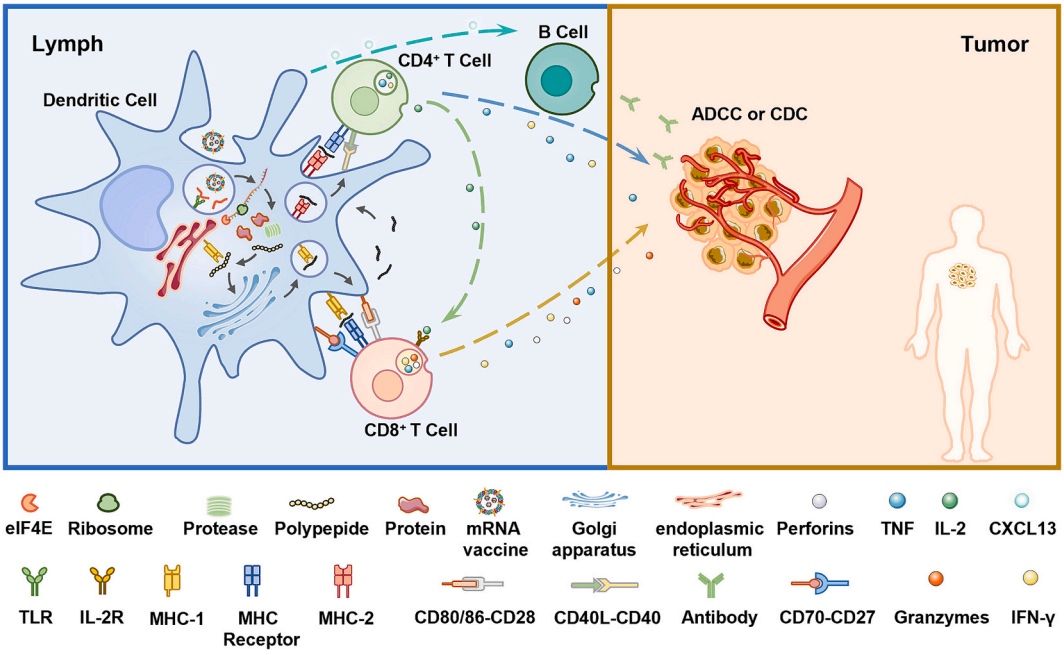

Fig. 3. The action mechanism of cancer mRNA vaccines.
After endocytosis of LNPs-encapsulated mRNA vaccine into cells, endogenous anionic lipids destroy the delivery complex by binding to cationic lipids and release mRNA into the cytoplasm. Binding of TLR (TLR3/TLR7/TLR8) to the endocytosed mRNA triggers the production of IFN- γ. Subsequent recognition of mRNA by eIF4E initiates mRNA translation. After mRNA is translated in the cytoplasm, the synthesized protein is hydrolyzed into peptides protease, loaded on MHC-I molecules, and delivered to the cell membrane surface through secretory pathway. MHC-antigenic peptide T cell receptor (TCR) complex and homologous receptor-ligand pairs (CD80/86; CD70/27) interact to activate $CD8^+$T cells. Activated $CD8^+$T cells secrete IFN-γ, TNF, granzyme and perforin, which act on tumor cells. In addition, the secreted antigens may be re-internalized and enter the MHC-II presentation pathway, and then TCR complex and CD40/CD40L co-activate $CD4^+$T cells synergically. In addition, $CD4^+$T cells secrete TNF to act on target cells. At the same time, IL-2 secreted by $CD4^+$T cells promotes the proliferation of $CD8^+$T cells which further assist the direct killing of tumor cells. Furthermore, activated T cells and follicular DCs (fDCs) jointly promote the production of plasma cells and memory B cells, which kill tumor cells through ADCC or CDC.

COVID-19 mRNA vaccines designed by Moderna and Pfizer-BioNTech uses pseudouridine instead of uridine to reduce the immune response [113]. Advances in delivery vehicles also better protected mRNA from degradation. In combination with ICIs, mRNA vaccines have greatly improved survival and cure rates in cancer patients. During the treatment of triple-negative breast cancer, mRNA vaccine in conjunction with a CTLA-4 monoclonal antibody significantly enhanced the anti-tumor immune response [46].

6. Conclusion and perspectives

With the development of molecular biology, mRNA vaccines have brought unprecedented hopes for cancer immunotherapy. Historically, the use of mRNA vaccines has been limited by instability, innate immunogenicity and low efficiency of in vivo delivery. Improvements in mRNA structure (e.g., codon optimization, nucleotide modification, self-amplifying mRNA; etc.) and delivery vehicles (e.g., LNPs, polymers, peptides;etc.) have largely overcome these barriers. Compared to other cancer vaccines, mRNA vaccines stand out for several reasons: (1) An mRNA vaccine can encode multiple antigens simultaneously which bind to both MHC-I and MHC-II to promote humoral and cellular immune responses. (2) Compared to DNA vaccines, mRNA vaccines are non-integratable, therefore free of mutagenic risk. (3) mRNA produced by IVT is free of pathogenic viral components and with no potential of infection. (4) Cancer mRNA vaccines offer the advantages with low costs, rapid production, and convenient renewal.

However, the mRNA field still faces challenges in terms of immunogenicity and effectiveness. Systemic inflammatory response may be the main concern for mRNA cancer vaccines, as mRNA inherently possesses immunostimulatory functions by activating the TLR7/8 pathway and inducing an IFN-I response. Currently, the innate immune response mediated by IFN-I can be largely reduced by removal of dsRNA contaminants, codon optimization and nucleotide modification. Another challenge is to determine the most feasible administration route for the vaccine. The administration route dictates the distribution of mRNA and influences the vaccine's efficacy. Muscle injection is a common and viable administration route, currently, FDA-approved SARS-CoV-2 mRNA vaccines are delivered through muscle injection [114]. However, intravenous injection allows mRNA to reach many lymphoid organs, and this administration method has been proven to stimulate a robust $CD8^+$ T cell anti-tumor immune response. Therefore, intravenous injection is the most commonly used direct administration route in mRNA cancer vaccine trials.

Personalization is the current direction of development for cancer mRNA vaccines. Therefore, the identification of mRNA sequences for specific target antigen is a current research priority. Using mass spectrometry, next-generation sequencing, and bioinformatics methods, scientists have predicted some novel epitopes that can bind to MHC-I molecules [115]. However, certain potential tumor-specific antigens (TSAs), such as gene fusion products or proteins generated by translation errors, still cannot be predicted by bioinformatics tools [116].

Most cancer mRNA vaccines are therapeutic rather than prophylactic, they require adequate vaccine potency when used as monotherapy, and require multiple administrations to induce an anti-tumor immune response. Single cancer mRNA vaccine therapy can be an effective treatment when it is used to treat patients with early-stage cancer, but is not applicable to patients with advanced cancer, in which the tumor microenvironment is highly immunosuppressive. Therapeutic cancer mRNA vaccines are more likely to be successful when used in combination with other immunotherapeutic approaches (e.g., ICIs, oncolytic virus, and adoptive cell therapy). (Table 2, NCT03897881, NCT04534205).

Prophylactic cancer mRNA vaccines also hold great promise. According to a review by Wen Xie et al. published recently in Nature Reviews in September 2021, the global mRNA prophylactic vaccine market size is projected to reach $12–15 billion by 2035 [117].

With the advancement of multi-omics and the integration of cross-disciplines, the screening of tumor-specific antigens will become more and more precise in the future. At the same time, patients with different cancer types or stages will be treated with appropriate combination therapies to achieve truly effective personalized treatment. Furthermore, we hope that increasingly prophylactic cancer mRNA vaccines will be available, so that we can truly prevent cancer before it occurs.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors give consent for the publication of the manuscript in BBA-Reviews on Cancer.

Availability of data and materials

Not applicable.

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Authors' contributions

YXL contributed to drafting and editing the manuscript and figs. WX, CMF, ZYZ designed, revised, and finalized the manuscript. QJY participated in the drafting and revising. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no competing interests.

Data availability

No data was used for the research described in the article.

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