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COMMENTARY

RNA cancer vaccines: developing mRNA nanovaccine with self-adjuvant property for cancer immunotherapy

Hongxia Zhang and Xiaojun Xia

Department of Experiment Medicine, State Key Laboratory of Oncology in Southern China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-sen University Cancer Center, Guangzhou, China

ABSTRACT

Messenger RNA (mRNA)-based cancer vaccine has become a popular approach for developing personalized and effective antitumor immunotherapy. To achieve robust antitumor efficacy, mRNA-encoding tumor antigens needs to be efficiently delivered and translated in dendritic cells for efficient antigen presentation; meanwhile, the vaccine would have adjuvant effect by stimulating innate immune response to boost the full activation of adaptive immunity. Recently, we reported a minimalist nanovaccine by formulating tumor antigen-encoding mRNA with a lipid-like material named C1, which could efficiently deliver mRNA into dendritic cells with simultaneous Toll-like receptor 4 (TLR4) stimulation, together induced T cell activation. Importantly, C1 mRNA nanovaccine exhibited significant antitumor efficacy on several tumor mouse models. Here, we discuss the nanovector-facilitated mRNA delivery and translation in dendritic cells, the self-adjuvant property of nanovectors, the challenges of personalized tumor antigen selection, and the potential strategies for developing efficacious mRNA cancer vaccines targeting the immunosuppressive tumor microenvironment.

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Cancer immunotherapies such as immune checkpoint blockade antibody and genetically engineered T cell therapy have made significant breakthrough in clinical practice.[1] Among these therapeutic modalities, a therapeutic cancer vaccine named Provenge has been approved by the FDA for metastatic prostate cancer treatment early in 2010, but the clinical efficacy was limited.[2] How to induce potent antitumor immunity in tumor microenvironment is still a major challenge for the development of therapeutic cancer vaccine. Last year, the whole world has witnessed the rapid development and powerful efficacy of mRNA-based vaccine for preventing COVID-19 virus infection. Both the BioNTech and the Moderna mRNA COVID-19 vaccines approved by the FDA worked by using mRNA encoding the Spike protein of the coronavirus to trigger the immune response.[3] Notably, a few years before the COVID-19 pandemic, the two companies already developed mRNA vaccines and carried out clinical trials for cancer treatment.[4,5]

The mRNA technology has been available for years. However, due to the instability and inefficient delivery of mRNA in vivo, the clinical application of mRNA drugs was challenging. In 2005, Kariko et al. found that lack of nucleoside modification in bacterial or in vitro synthesized mRNA molecules rendered them recognized by TLRs, followed by inflammatory and antiviral responses, which may cause mRNA translation inhibition and degradation.[6] Nucleoside modification such as pseudouridine or 2′-thiouridine would greatly reduce the immunogenicity of IVT mRNA and thus facilitate in vivo mRNA translation.[7,8] In addition to nucleotide modification, nanoparticle-based vectors also greatly improve the in vivo delivery and stability of mRNA.[9] For example, the two FDA-approved COVID-19 mRNA vaccines both used lipid nanoparticles for mRNA formulation.[3,10] Even so, both vaccines still require very low temperature for transportation and storage. In comparison, another China-based mRNA vaccine was shown to be stable for at least one week at room temperature by lipid nanoformulation in preclinical study.[11] Thus, more advanced nanovectors and mRNA modification technologies are desired for manufacturing mRNA vaccine with less demanding storage requirement and better in vivo efficacy.

The in vivo target cell population for vaccine delivery is mainly dendritic cells (DCs), as they are generally considered the most professional antigen presenting cells.[1] Ideally, the nanovaccine applied would go dendritic cells for antigen expression and presentation. If mRNAs are expressed in normal cells, these cells may become target cells for vaccine-induced T cells, and potentially cause side effects such as autoimmune attack. The side effect of COVID-19 vaccine may be from such off-target mRNA delivery and expression.[12] By optimizing the surface charge of lipoplex materials, Kranz et al. reported a spleen-enriched lipid nanoparticle that targeting DCs for mRNA delivery, and exhibited efficient antigen expression and immune activation with limited toxicity. The RNA-lipoplexes were effectively targeted to the spleen with intravenous injection [13]. Thus, targeted delivery either by modifying the size, surface charge and chemistry of nanovector, or conjugate a targeting moiety, such as antibody recognizing certain surface marker on dendritic cells, may increase the vaccine efficacy and reduce side effect.[13] Administration route is another factor to consider for DC-

CONTACT Xiaojun Xia xiaxj@sysucc.org.cn Department of Experiment Medicine, Sun Yat-sen University Cancer Center, 651 Dongfengdong Road, Guangzhou 510060, China.

targeting delivery. For example, Lindsay et al. demonstrated that when administrated by intramuscular injection, professional antigen-presenting cells were the primary cells containing injected mRNA at the injection site and in the draining lymph nodes [20]. Currently, many groups are optimizing nanoformulations and administration routes to improve in vivo efficacy of mRNA vaccines.

To identify nanomaterials suitable for mRNA vaccine vectors, we set up an in vitro DC-based vaccine screening system by adapting an in vitro antigen presentation assay.[14] In this system, DCs were primed with Ovalbumin (OVA)-encoding mRNA nanovaccine, followed by co-culture with OVA-specific T cells. The activation level of T cells, reflected by cytokine IL2 or IFNγ production, serves as the readout of vaccine efficacy. Lipid-like materials have been previously used for small interfering RNA delivery, but whether they can serve as mRNA vector is not well studied. We then screened a library of cationic lipid-like compounds that were efficient ring-opening of epoxides by generation 0 of poly(amidoamine) (PAMAM) dendrimers by antigen presentation assay in vitro.[15] The top candidate was the lipid-like material C1 with a 12-carbon tail that could effectively deliver mRNA into DCs, produce efficient mRNA translation and induce robust T cell response.[15] Notably, previous studies used lipid-like material D2 in this library known as G0-C14 for siRNA delivery,[16–18] but our result showed that D2 was not suitable for mRNA delivery. This may reflect the different chemistry and binding affinity of double-stranded short siRNA versus single-stranded mRNA. By testing C1 formulated with specific cancer antigen-encoding mRNAs on different tumor models including MC38 colorectal tumor and B16 melanoma models, we also observed robust immune responses and antitumor efficacy of C1-mRNA vaccine in both tumor prevention and therapeutic settings with no obvious toxicity. Mechanistically, we found that C1 nanoparticles stimulated TLR4 signaling in mouse bone marrow dendritic cells (BMDCs) and significantly induced the expression of proinflammatory cytokine genes IL-1β, IL-6, IL-12, and type I interferon genes in a mild magnitude.[15] Therefore, C1 serves as a self-adjuvant as well as mRNA delivery vector. TLR4 is essential for C1-mRNA-induced immune activation and in vivo antitumor efficacy, as the antitumor efficacy of C1-mRNA was completely abolished on *Tlr4*$^{-/-}$ mice.[15]

Although we have identified C1 as a new mRNA nanovector with self-adjuvant property, there are still questions need to be further explored. The first question is how to select tumor antigens. Unlike vaccines for virus with defined "foreign" antigens, cancer vaccines often use tumor-associated antigens that are highly expressed on tumor cells, or neoantigens derived from tumor mutation.[19] The FDA-approved cancer vaccine Provenge used DCs expressing a single tumor-associated antigen, PSMA, fused with GM-CSF for metastatic prostate cancer treatment.[2] Thanks to the advances of DNA sequencing technology and antigen prediction algorithm, tumor mutation information can be obtained within a few days after tumor tissue isolation.[20] The potential neoantigens were predicted by computational algorithm, then the coding sequences for these predicted antigens can be constructed into DNA vectors as the template for in vitro mRNA synthesis, followed by mRNA/nanovector formulation and in vivo application.[5] However, the accuracy of the current prediction algorithm is still very low. Besides class I antigens, class II antigens are also important for eliciting effective antitumor immunity.[21] Due to the longer peptide length and more flexible binding with MHC-II molecules, class II antigens are even harder to predict. The advances in proteomic technology to directly identify peptides bound on MHC molecules may facilitate the search for tumor-specific antigens.[20] Thus, precise tumor antigen identification and selection is still a pressing question need to be solved for successful personalized mRNA cancer vaccine development.

Another question is how to incorporate the immune adjuvant component into mRNA vaccines without interfering mRNA translation. Vaccines often contain an adjuvant component to help elicit desired T- or B-cell responses by stimulating innate immune responses.[22] Exogenous single-stranded mRNA molecules are identified as a PAMP (Pathogen-associated molecular pattern) when delivered to cells. Single-stranded oligoribonucleotides and their degradation products are detected by the endosomal sensors Toll-like receptor 7 (TLR7) and TLR8,[23,24] resulting in type I interferon (IFN-I) production. Early studies have found that activation of inflammatory responses or IFN-I response by native mRNA would induce an antiviral state, which leads to reduced mRNA translation and mRNA degradation.[6,7] Thus, the immune-boosting effect of an adjuvant and efficient translation of antigen-encoding mRNA seems a paradox for mRNA vaccine. Nevertheless, recent studies using lipid nanoparticles with self-adjuvant property for mRNA vaccine formulation seemed to circumvent this problem. Several studies reported that antiviral signaling activation contributed to the antitumor efficacy of mRNA nanovaccine delivered with specific lipid nanoparticles.[17,25] In a recent study, Miao et al. reported an mRNA vaccine delivered by heterocyclic lipids induced antigen presenting cells maturation via STING (stimulator of interferon genes) pathway activation and resulted in enhanced antitumor efficacy.[26] Similarly, a minimalist nanovaccine comprising antigen and PC7A (a pH-sensitive polymer bearing a seven-membered ring with a tertiary amine) nanoparticle generated a strong cytotoxic T cell response with STING activation.[27] More recently, the authors further demonstrated that PC7A could stimulate the prolonged production of pro-inflammatory cytokines by binding to a noncompetitive STING surface site.[28] By using TLR4-deficient and STING-deficient mouse models, we have found that the immune-stimulating effect of C1-mRNA is largely dependent on TLR4 but not STING.[15] Either excess type I interferon response or pro-inflammatory cytokine production potentially cause systemic toxicity, while C1-mRNA nanovaccine-induced innate immune activation did not show obvious toxicity in vivo.[15] It is possible that lipid-like material C1 is a mild TLR4 agonist like MPLA (Monophosphoril lipid A), and elicits a response adequate for dendritic cell activation but not systemic inflammation.[29] In fact, C1 induced a much lower level of cytokine production than LPS in mouse sera. Further characterization of innate immune signaling is warranted to delineate the underlying mechanisms.

Due to the complicated etiology and tumor heterogeneity, it is difficult to design prophylactic cancer vaccines to prevent cancer development except for a few virus-related cancer types, such as HPV-related cervical cancer or HBV-related liver cancer. Therefore, most cancer vaccines serve for

therapeutic purpose.[30] The tumor tissues consist of many types of cells in addition to cancer cells, including fibroblast, immune cells, endothelial cells, etc. By the time of diagnosis, the immune surveillance has already failed to control the tumor growth; to make it even worse, tumor cells have evolved to cooperate with other cells in the tumor tissue to form an immunosuppressive microenvironment facilitating immune escape.[19] Thus, it would be more challenging for cancer vaccines to reverse immune suppression and induce cancer-specific immunity. Normalization of the tumor microenvironment, including tumor vasculature and immune infiltration, and functional recovery of effector T cells, is required for effective immunotherapy.[31] Thus, combination therapy using mRNA vaccine and therapeutics targeting tumor microenvironment is a promising approach on clinical trials. For example, anti-PD1 was used together with mRNA vaccine or peptide-based vaccine to boost the antitumor efficacy in the clinical trials treating melanoma patients, and both showed effective and durable clinical response.[5,32]

Overall, mRNA cancer vaccine is rapidly advancing in both preclinical development and clinical trials. Nanovectors such as TLR4-stimulating lipid-like materials or STING-activating cyclic lipid nanoparticles not only facilitate mRNA delivery into dendritic cells, but also serve as self-adjuvant to activate the antigen presentation function of DCs, thereby potentiating antitumor immunity.[15,26,27] Precise identification of tumor antigen would be a prerequisite to developing successful personalized mRNA vaccine, and current progress in genomic sequencing and proteomic technology would greatly improve the accuracy of tumor antigen identification. Combination therapy using mRNA vaccine, tumor angiogenesis inhibitors, and checkpoint blockade antibodies, such as anti-PD1 would facilitate tumor immune infiltration and sustain the effector function of vaccine-elicited antitumor immunity in the tumor microenvironment, and potentially lead to complete response and long-term disease control.

Disclosure of potential conflicts of interest

The authors report no conflicts of interest.

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ORCID

Xiaojun Xia http://orcid.org/0000-0003-4444-7472

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