Application of mRNA Technology in Cancer Therapeutics

Subjects: Oncology

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mRNA-based therapeutics pose as promising treatment strategies for cancer immunotherapy. Improvements in materials and technology of delivery systems have helped to overcome major obstacles in generating a sufficient immune response required to fight a specific type of cancer. Several in vivo models and early clinical studies have suggested that various mRNA treatment platforms can induce cancer-specific cytolytic activity, leading to numerous clinical trials to determine the optimal method of combinations and sequencing with already established agents in cancer treatment.

Keywords: mRNA vaccines ; mRNA delivery platforms ; cancer therapeutics

1. Cancer Immunology and Immunotherapy

The immune system comprises of elements of myeloid and lymphoid lineage such as lymphocytes and macrophages, which are specialized to generate an immune response against foreign-appearing structures in the host, including cancerous cells. When a tumor cell encounters the innate immune system, an inflammatory signaling cascade is initiated, which stimulates induction of dendritic cell maturation, immunostimulatory cytokine secretion, and natural killer cell activity. Once tumor cells are internalized by dendritic cells, these APCs interact with the microenvironment to present the neoantigen to cytotoxic T cells and B cells, which are subsequently activated to develop an antigen-specific immune response. The generation of an active adaptive immunity requires dendritic cell maturation and induction of danger signals from both apoptotic or necrotic cells and the TME during antigen processing [1][2]. Nevertheless, as tumors progress, the anticancer immunologic activity may be hampered by the upregulation of various checkpoints and immune-suppressive elements of the host immune system, which are naturally programmed to balance any excessive immune activity against the host. Recently identified inhibitory immunoreceptors, such as lymphocyte-activation gene 3 (LAG-3) and T cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT), indoleamine 2,3-dioxygenase (IDO) controls immune activity by suppressing tumor-specific T and B lymphocytes, resulting in a shift towards generation of an immune-suppressive stroma comprising of exhausted T cells, which lack the ability to generate an anti-tumor immune response; as well as myeloid and lymphoid elements of the immune system associated with immune escape, namely regulatory T cells (Treg), immature dendritic cells, M2 macrophages, and myeloid derived suppressor cells (MDSC) [3]. It has been shown that cancer cells tap into the host immune system to overcome immune-mediated cell killing by switching the tumor microenvironment to an immunosuppressive or immune-cold phenotype mediated by several cytokines and molecules [3][4][5].

Immunotherapy refers to all types of treatment strategies aiming to restore immune dysregulation or to modulate the host immune system to destroy cancer cells. The current immunotherapy approaches involve strategies that aim to release the brakes on the host immune regulatory systems by inhibiting checkpoint upregulation by programmed cell death protein-1 (PD-1), programmed cell death ligand-1 (PD-L1), or cytotoxic T lymphocyte antigen-4 (CTLA) inhibitors, or to stimulate the immune system to generate a cancer-specific response by cancer vaccines or to administer ex vivo activated autologous or allogeneic immune cells that target cancer cells, such as chimeric antigen receptor-T (CAR-T) cells or engineered natural killer (NK) cells, otherwise referred to as adoptive cell therapy [6][7][8][9][10][11].

2. The Evolving Role of mRNA Technology in Cancer Immunotherapy

Nucleic acids in the form of RNA or DNA, whether exogenous from bacterial or viral causes, or endogenous, shed from cancer cells are capable of inducing variable degrees of immune response [12]. Cancer antigens, whether as whole-cell lysates, peptides, or as nucleic acids intended to translate into the structural protein of the antigen itself, can be delivered into the host in order to generate a cancer-specific immune response, otherwise referred to as a “cancer vaccine”. In

contrast to peptide-based or whole cell vaccines, nucleic acid vaccines are more advantageous since they enable delivery of multiple or full-length tumor antigens, leading to a broader immune response.

In vitro transcribed RNAs, which are proven to have a wide applicability in SARS-CoV-2 vaccinations, have recently gained interest as cancer vaccines due to their versatility to encode chimeric peptide structures, allowing for targeting cancer cells with diverse and complex mutational structures. Furthermore, mRNA vaccines have emerged as an appealing alternative to DNA vaccines not only due to their ability to be translated in both dividing and nondividing cells, but also due to their safety since they cannot integrate into the host genome [13].

As synthetic mRNA enters the host cells through the cell membrane or by endocytosis, translation to the peptide of interest occurs within the cytosol. This protein structure, which is undistinguishable from the product of endogenous mRNA, undergoes post-transcriptional modifications eventually leading to degradation by intracellular compartments. These peptides are then presented on major histocompatibility complexes (MHC) of the antigen-presenting cells to be introduced to the effector cells of the host immune system to induce cancer-specific killer T cells along with activated helper T lymphocytes and NK cells [14]. In addition to the generation of a cancer-specific immune response, exogenous mRNA helps to maintain an immune-friendly tumor microenvironment (TME) by triggering secretion of type I Interferon (IFN) and other inflammatory cytokines through activation of toll-like receptors (TLR) and retinoic acid-inducible gene I (RIG-I) [15]. Furthermore, mRNA constructs can be engineered to express proinflammatory cytokines including, but not limited to Interleukin 2 (IL-2) IL-7, IL-12 and IL-15, which act synergistically to enhance generation of antigen-specific CD 8 + cytotoxic T cells, increase the ratio of active CD8 cells to immune suppressive Tregs, and induce memory T cells for a long-lasting immune response [16][17][18][19][20]. In addition, mRNAs are also being developed to encode monoclonal antibodies (mAbs), which have an established role as a passive targeted immunotherapy approach for various cancer types such as Her-2 positive breast cancer and lymphomas. There is in vivo evidence that suggests mRNA encoded mAbs are indeed able to induce a more sustained antitumor effect as compared to their recombinant equivalents in murine models [21][22]. These constructs can be modified to encode bispecific mAbs comprising an anti-CD3 Fv and a tumor-specific Fv, which are able to redirect T cells to the TME to elicit a stronger immune-mediated tumor cell killing [23].

3. The Immunogenicity and Molecular Biology of mRNA-Based Immunotherapy

Therapeutic mRNAs are produced through in vitro transcription (IVT) catalyzed by DNA-dependent RNA polymerase, which selectively recognize the promoter region of DNA templates [24]. The end product is a naked mRNA strand, which should be modified to optimize the stability and translational ability. These modifications include capping the 5′ end, optimizing the sequence of the untranslated translating regions, and adding a poly-A tail [25]. Nevertheless, these alterations and byproducts generated during the IVT process may impede the desired antitumor response through activation of the innate immune system, leading to the recognition of the modified mRNA molecules as nonself, as well as interference with the transcriptional capacity by cellular stress mechanisms [26].

As the first line of defense against external and internal pathogens, the innate immune system initiates a cascade of events subsequently triggering adaptive cancer-specific immunity. Endogenous and exogenous non-self-nucleic acids are recognized by intracellular pattern recognition receptor family (PRR) comprising TLRs, RIG-I-like receptors, nucleotide-binding and oligomerization domain (NOD)-like receptors, C-typelectin receptors, absent-in-melanoma 2 (AIM2)-like receptors, and the cyclic GMP-AMP synthase. Activation of PRRs localized in the cytosol and endosomal compartment in turn lead to transcriptional activity of several proinflammatory cytokines and chemokines, and stimulation of transcription-independent intracellular pathways such as autophagy, apoptosis, and phagocytosis. Studies with synthetic nucleic acids to manipulate the immune system have shown that different sequences of dsRNA (siRNA) varying in length induce distinct immune responses, which may be in opposite directions [12][27][28]. Therefore, purity and nucleotide composition of therapeutic mRNAs play a significant role in generating an optimal immune response.

3.1. mRNA Vaccine Structure

Two types of mRNA-based vaccines are available: nonreplicating (NRM) and self-replicating mRNA (SRM) vaccines, which are composed of a universal 5′ cap, 3′ and 5′ noncoding regions, an open reading frame, and a 3′ poly-A tail. While the cap structure protects the mRNA from quick degradation and induces IFN-mediated immune responses, the untranslated regions regulate the translational efficiency of mRNA. The poly A tail plays a significant role in the translation by regulating the stability of mRNA. Enrichment of G:C content and utilizing modified codons in the ORF constructs and optimizing the length of the poly-A sequence are some of the structural modifications that promote a translational process [29][30][31][32]. The NRM, though technically less demanding to produce, has the disadvantage of limited activity and

stability, which can be overcome to a certain extent by structural optimization. SRM vaccines differ from NRMs by including an extra construct that encodes a replicase component. Generally, these vaccines are produced through engineering of single-strand RNA viral structural genes, which have been substituted by the gene of interest (i.e., cancer antigen), while keeping the nonstructural genes (i.e., replicase), leading to a high level of antigen expression within a delivery system. Picornaviruses, alphaviruses, and flaviviruses are the most common RNA viral systems employed to generate SRM vaccines [33][34][35][36][37].

3.2. mRNA Delivery Platforms

Although mRNA technology is a promising tool for cancer immunotherapy, a number of challenges have to be faced to facilitate an effective immune response. First, the large and negatively charged RNA molecule has to cross the cell membrane, which is a significant barrier to intracellular delivery due to its negative charge. Once mRNA enters the cell, there is a high risk for degradation through ribonucleases, which are abundant throughout the skin and systemic circulation. Although delivery of naked mRNA is possible through intradermal, subcutaneous, and intramuscular routes, the efficacy of such approaches is hindered by a short half-life, rapid degradation and inadequate immune response due to ineffective access to intracellular compartments. Therefore, an efficient delivery is crucial to achieve favorable therapeutic potential. Therapeutic advances in mRNA technology have been linked to the development of various nanotechnological delivery systems that have been engineered to ensure optimal translational capability [13][33][38].

3.2.1. Synthetic Systems

Lipid-based Delivery Systems

Lipid-based materials are the most extensively investigated delivery systems for RNA-based therapeutics. Referred to as lipid nanoparticles (LNP), these structures consist of a cationic or more recently a pH-dependent ionizable lipid layer; a polyethylene glycol (PEG) component; phospholipids and cholesterol [38][39]. The ionizable amino lipid layer is designed to obtain a positive charge as pH drops, facilitating endosomal uptake of the liposome, while retaining encapsulation of the negatively charged mRNA molecule. The PEG molecule plays a significant role in preventing macrophage-mediated degradation, together with providing stability along with cholesterol [40][41][42]. The structure of the amino lipid component plays a key role in delivery efficacy, tolerance, and tissue clearance [43]. Efforts to optimize LNP delivery of mRNA vaccines have yielded efficient RNA delivery in cell lines, and strong, long-lasting humoral immune responses against several viral pathogens in murine models [44][45][46]. Clinical studies with two LNP-based mRNA vaccines against the SARS-CoV2 virus during the pandemic have confirmed the favorable results, showing a strong efficacy across different populations, leading to regulatory approval despite the short-term follow-up period [47][48].

Nevertheless, as part of a cancer vaccine, LNP design should further be developed to deliver the mRNA cargo specifically to antigen-presenting cells, while preventing degradation and retain effective translational capacity. Moreover, the amino lipid structure should be biodegradable to prevent toxicity and allow for multiple dosing at the same time. Emerging evidence from preclinical studies suggest that LNP mRNA vaccines provide robust antigen-specific antitumor with memory T cell responses by specifically targeting dendritic cells, leading to prevention of tumor growth in murine models [49][50][51][52].

2. Polymer-based Delivery Systems

Polymeric materials and dendrimers, modified with nanotechnologic fatty side chains to reduce toxicity and avoid enzymatic degradation in vivo, have gained popularity to deliver mRNA as vaccines against fatal viral pathogens such as HIV, Zika, Ebola, and H1N1 Influenza [53][54][55][56][57]. Polymeric structures surrounded by a PEG outer shell have been used in murine models to deliver an antiangiogenic RNA sequence, which was shown to inhibit growth in a pancreatic cancer model [58]. Similarly designed mRNA vaccines have been shown to effectively translate into tumor-associated antigens in vivo [59]. Furthermore, a polymer-based RNA vaccine encoding PTEN has successfully been introduced into several castration-resistant prostate cancer models and has been shown to inhibit tumor growth by restoring PTEN function [60].

3. Peptide-based Delivery

Cell-penetrating peptides (CPPs) are cationic peptides that can translocate through the cell membrane independent of receptors and can transport proteins, small organic molecules, nanoparticles, and oligonucleotides. Because of a favorable safety profile and efficient transfection capability, CPPs represent a promising class of nonviral delivery vectors [61][62][63]. Nevertheless, low cell and tissue selectivity, and impaired internalization of the cargo by conjugation through

different cellular layers limit efficient clinical application [64]. Recent efforts have been focused on identifying the most optimal CPP for enhanced immune activity. Protamine is a cationic peptide that can prevent lysosomal degradation during delivery of RNA. Protamine-based deliveries have been shown to induce a strong immune response through toll-like receptor 7 activation [65][66]. More recently, advances in biotechnology have led to promising developments in peptide-based mRNA delivery. For example, a pegylated cationic KL4 peptide complex in powder form has been successfully used as an aerosolized delivery system for pulmonary delivery [67]. Furthermore, an optimized GALA-peptide conjugated mRNA encoding the Ova peptide exhibited a strong APC uptake and an efficient endosomal escape, leading to enhanced antigen-specific T cell activity and dendritic cell maturation compared to naked RNA or different peptide complexes [68].

3.2.2. Biological Systems

- Ex Vivo Transfected Cellular Systems

Immunotherapy against cancer requires transfection of APC with specific antigens or nucleic acids such as mRNA, which translate into tumor-specific antigens. Although in vivo transfection via the intramuscular, intravenous, or subcutaneous routes are possible, the immune response generated is usually weak and unsustained. Therefore, ex vivo transfected engineered dendritic cells or chimeric antigen receptor T cells have been developed as cancer vaccines or adoptive cell therapy strategies to target cancer cells once introduced in the host [69].

- Dendritic Cells

Dendritic cells play a crucial role in reprogramming the immune system by their ability to uptake and present the tumor antigens, leading to generation of potent effector cell activity directed against cancer cells. Additionally, mature dendritic cells are capable of modulating chemokine- and cytokine-induced lymphoid activation, which are strictly relevant for a systemic and sustainable anticancer immune response. As autologous cancer vaccines, dendritic cells are harvested from the host by apheresis, isolated from mononuclear cells or progenitor stem cells, subsequently stimulated by various cytokines to achieve maturity, followed by transfection with specific antigens as nucleic acids or peptides. Numerous efforts have been focused on methods to achieve a stronger immune response through more efficient antigen presentation, migration to required lymphatic tissues, and induction of a stronger cytokine production through generation of Notch differentiated dendritic cells with engineered receptor expression capability using clustered regularly interspaced short palindromic repeats (CRISP-R) gene editing and RNA interference, as well as the use of optimized maturation cocktails [70][71][72]. Ex vivo transfection of mRNA-loaded dendritic cell vaccines against a variety of tumor specific antigens such as telomerase reverse transcriptase (TERT) and the melanoma cell line B16F10 have led to generation of a strong antitumor immune response in murine melanoma models [73][74].

b. CAR-T Cells

Chimeric antigen receptor (CAR)-modified T cells represent a novel adoptive cell therapy approach that has been shown to effectively target tumor cells leading to a potent immune-mediated cancer cell killing [75]. CAR-Ts confer several advantages over natural host immunity by MHC independent tumor antigen presentation, more potent cell receptor binding, and ability to bypass escape mechanisms such as HLA downregulation [76]. Direct transfection by electroporation or viral systems has been utilized to deliver CAR-encoding mRNA to generate cancer-specific CAR-T cells [77]. More recently, RNA optimization by nanoparticles and gene editing through CRISP-R technology has been utilized to engineer CAR-Ts that have improved stability and transfection ability [78][79]. Preclinical studies investigating ex vivo transfection of patient-derived T cells by retroviral constructs to deliver mRNA encoding bi-CARs targeting tumor-specific epitopes have shown that the engineered CAR-Ts are capable of recognizing target antigens and overcoming escape variants, eventually leading to improved survival in a glioblastoma (GBM) murine model [80]. Furthermore, profound cytotoxic cell lysis has been demonstrated with RNA transfected CAR-T constructs expressing CD19 in xenograft models with leukemia [81][82], extensively reviewed elsewhere by Rajan et al. [83].

2. Viral Constructs

Viral constructs generated from RNA viruses have been evaluated extensively as self-replicating RNA (SRM) vaccines against several cancer types. Single-strand RNA viruses including alphaviruses, flaviviruses, and rhabdoviruses can be engineered to form naked RNA replicons and recombinant viral-like particles (VLP), which are capable of producing a high level of tumor antigen expression in APCs, in turn leading to a strong immune response [84][85]. An SRM vaccine comprises a replicon carrying the gene of interest in conjunction with the replicase gene and a defective virus encoding structural genes, forming VLP in a packaging cell construct. The VLP, taken up by APCs when introduced into the host, deliver self-replicating RNA constructs to the cytosol by receptor-mediated endocytosis, leading to a high level of RNA

production and tumor–antigen expression through translation [37][86][87]. Preclinical studies evaluating the role of replicon-based SRM vaccines have shown the success in eliciting strong humoral and cellular immune responses against several cancer types in xenograft models harboring Her-2 neu breast cancer, prostate cancer, GBM, and human papilloma virus (HPV)-induced tumors [88][89][90][91].

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