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REVIEW

RNA vaccines for anti-tumor therapy

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Abstract

The immune system is able to recognize tumor antigens and this has been the basis for the development of cancer immunotherapies. The immune system can be instructed to recognize and attack tumor cells by means of vaccination strategies. One such strategy involves the delivery of tumor antigen as genetic material. Herewith we describe the use of RNA encoding tumor antigens for vaccination purposes in tumor settings. RNA has features that are interesting for vaccination. Upon transfection, the RNA has no possibility of integration into the genome, and the tumor translated proteins enter the intrinsic antigen processing pathway thus enabling presentation by MHC-I molecules. This can specifically activate cytotoxic CD8 T cells that can attack and kill tumor cells. RNA can be delivered as a naked molecule for vaccination purposes or can be used to transfect dendritic cells. The combination of RNA technology with dendritic cell vaccination provides a powerful tool for cancer immunotherapies.

Key words: RNA; Vaccine; Dendritic cells; Immunization; Cytotoxic T cells; Tumors

Core tip: In this review we discuss the use of RNA encoding tumor antigens for anti-tumor vaccination. RNA has several features that makes it relevant for vaccination purposes. Importantly, the RNA has no possibility of integration into the genome, and the tumor translated proteins enter the intrinsic antigen processing pathway thus enabling presentation by MHC-I molecules thus specifically activating cytotoxic CD8 T. Further, RNA can be delivered as a naked molecule f or can be used to transfect dendritic cells. This combination of RNA technology with dendritic cell vaccination provides a powerful tool for cancer immunotherapies.

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TUMOR IMMUNOLOGY

Cancer is one of the leading causes of mortality in humans and most of the successes obtained battling this disease rely on early prevention even though a gamut of treatments such as chemotherapy, radiotherapy and surgery are available to patients. In view of this situation it becomes necessary to generate innovative approaches for the treatment of this disease. One such strategy entails educating the immune system to recognize and destroy tumor cells. To this end, several immunotherapeutic strategies have been designed and tested in preclinical studies and clinical trials.

Tumors are composed not only by cancer cells, but also by other cellular types such as fibroblasts, endothelial

cells and infiltrating leukocytes that together with extracellular matrix components constitute the microenvironment of the tumor[1]. In recent years the relevance of the tumor microenvironment as a key player in cancer progression has been highlighted and the role of its cellular populations and extracellular matrix components examined. In this context, immune cells play a double edge sword role[2].

On one hand, the protective role of the immune system against tumors has been widely described and indeed the presence of tumor-infiltrating lymphocytes (TILs) has been reported in numerous studies involving melanoma[3], colorectal[4-7], breast[8,9], ovarian[10-16], prostate[17], renal[18], and esophageal carcinoma[19]. These TILs are able to recognize tumors as demonstrated by their capability to get activated by tumor antigens and kill cancer cells *ex vivo*[10,20-22]. Notably, several reports showed that the prevalence of certain T cell populations is associated with a better outcome in different types of cancers. Particularly, studies involving ovarian, non-small cell lung, mesothelioma, colon, and urothelial cancers showed that a high CD8/regulatory T cell ratio among TILs is usually associated with a better prognostic or a better response to antitumor treatment[14,23-28].

On the other hand, the presence of a robust number of regulatory T cells within the TILs, or a CD4/CD8 ratio that favors CD4 T cells, has been associated with a worse outcome or tumor growth in various studies[29-33]. These studies highlight the ability of the immune system to recognize tumors and provide a rationale for pursuing immunotherapeutic approaches, but also underscore the hurdles for its success. Similarly, other tumor-associated leukocytes such as myeloid-derived suppressor cells (MDSCs) can promote tumor growth by modulating the immune response[34]. Indeed, we have previously demonstrated the relevance of the tumor microenvironment in attracting MDSCs by a complement-mediated process[35]. Further, the presence of a subset of splenic dendritic cells (DCs) with the ability to suppress antitumor T cells responses *via* indoleamine 2,3-dioxigenase expression highlights the immunosuppressive role of antigen presenting cells (APCs) in some tumor settings[36]. Notably, leukocyte infiltration can precede the development of a neoplasm, being chronic inflammation a risk factor for the development of cancer[37-39]. Further, inflammatory conditions such as caused by certain types of infections can be involved in the pathogenesis of many human malignancies. For example, gastric carcinomas can arise in a *Helicobacter pylori*-induced gastritis environment[38] or hepatitis B virus/hepatitis C virus can induce hepatocellular carcinomas[39]. Also, chronic but non-infective inflammatory conditions as in the case of smoking-related bronchial cancer can induce carcinogenesis[40]. In the same way, chronic pancreatitis is considered a risk factor for the development of pancreatic cancer, and many of the growth factors involved in tissue remodeling and regeneration in chronic pancreatitis are present in pancreatic cancer[41]. In addition, there is strong evidence that tumor-associated leukocytes can also promote tumor angiogenesis. In particular, infiltrating inflammatory cells secrete a diverse repertoire of growth factors and proteases that potentiate tumor growth by stimulating angiogenesis. We and others have described the capability of APCs such as DCs or macrophages, to collaborate with neoangiogenesis in human cancers and in different mouse tumor models[42-47]. Thus, tumors exhibit an arsenal of mechanisms in order to inhibit an effective immune response.

Collectively, these data indicate that in some settings immunoablative procedures must precede immunotherapeutic treatments. To this end, some studies have suggested that depletion of regulatory T cell populations or tumor-associated leukocytes can enhance the effectiveness of a subsequent immunotherapy[3,48].

TUMOR IMMUNOTHERAPY

The ability of the immune system to recognize and attack tumors relies on the presence of tumor-specific antigens (TSAs) and tumor-associated antigens (TAAs). As recently reviewed by Aly[49], TSAs are expressed only by tumor cells due to mutations in normal cellular genes, or to the expression of viral antigens or normally suppressed oncogenes in cancer cells. On the other hand, TAAs are molecules expressed both in normal and cancer cells but expressed at higher levels by tumors, or expressed by normal cells only during the embryonic state differentiation. For the purposes of the present review, tumor antigens will be named generically as TAAs.

Pioneering studies performed by Rosenberg *et al*[50] in melanoma aimed to activate lymphocytes *in vivo* by treating cancer patients with IL-2. The rationale being that the patients' T cells have the ability to recognize and attack tumors. Indeed, this is the basis for immunotherapies using TILs. To carry out these T cell adoptive therapies, upon purification from tumor tissues, TILs are expanded and activated *ex vivo* using TAAs and are subsequently re-infused into patients[51]. Recent advances in this area involve the generation of TAA-specific T cells by means of genetic recombination. As previously described in detail, chimeric antigen receptor (CAR) T cells are engineered to express the portion of an antibody that recognizes an antigen fused to the T cell receptor signaling region[52]. Thus, they recognize TAA on tumor cells with the specificity of an antibody and they kill them using the cytotoxic machinery of T cells[52]. This circumvents the problem of isolating TILs, which might not be present in all patients or present at very low numbers in tumor samples. Recently, by using CAR T cells, Kalos *et al*[53] were able to completely eradicate cancer cells in patients with advanced leukemia.

Additional immunotherapeutic strategies have been proposed and investigated based on the ability of the immune system to recognize TAAs. One such strategy involves inducing immune responses against TAA by means of vaccination. To this end, TAAs are used as tumor lysates, proteins purified from these lysates, or pep-

tides (derived from tumor protein digests or synthesized *in vitro*). Furthermore, as described below, tumor vaccination strategies also involve the use of apoptotic or necrotic tumor cells as way of delivering the TAAs. These molecules will be recognized *in vivo* by resident APCs, which are key components of the innate immune system. The innate immunity is the first line of defense against pathogens. Cells of the innate immune response include macrophages, granulocytes, DCs, and natural killer cells. Macrophages, B lymphocytes and DCs are generally described as APCs. After ingesting a pathogen, APCs are able to eliminate it through various mechanisms involving enzymatic degradation and the use of reactive oxygen or nitrogen species. APCs detect pathogens through the expression of pattern recognition receptors (PRRs) which are able to recognize conserved pathogen associated molecular patterns (PAMPs). Some of the main PRRs include membrane associated toll-like receptors (TLRs) and cytoplasmic NOD-like receptors[54,55]. DCs are highly effective APCs distributed throughout the body, particularly in immunological organs such as thymus, spleen, lymph nodes and Peyer's patches[56-58].

DC ACTIVATION PROCESS

Immature (non-activated) DCs present in peripheral tissues can detect PAMP-bearing microorganisms through their high expression of cell surface, vesicular and cytoplasmic PRRs[59]. This process leads to the activation of the DCs, which can degrade pathogenic proteins (both recovered from the extracellular space, or from the cytoplasmic pool) and process them into peptides[58]. Antigenic peptide fragments derived from the processed pathogen molecules are the exposed on the surface of the DCs in the context of MHC I or Ⅱ molecules. During this process, an immature DC will undergo "maturation" due to presence of inflammatory cytokines generated by the DC itself, or by other surrounding cells in response to the pathogen or tissular damage. This maturation process entails upregulation of MHC class Ⅱ molecules, costimulatory molecules such as CD40, CD80, CD86; OX40L and the chemokine receptor CCR7. This receptor recognizes the chemokines CCL19 and CCL21 which are constitutively expressed at high levels by lymph nodes[60]. Thus, mature DCs migrate from the sites of antigen capture to the T-cell regions of draining lymph nodes, where they contact naïve or memory T cells. Through interaction with specific cell receptors for antigen on the surface of T lymphocytes, DCs select and activate specific T cell clones with the capability to recognize the presented antigen[58,61,62]. In this way, DCs tie the innate and adaptive immunity, being keystones for the development of antigen specific immune responses.

APCs have different ways of processing and presenting antigens. Typically, antigens that are captured by the phagocytosis or endocytosis are degraded in the lysosomal compartment and peptides are presented by MHC-Ⅱ molecules on the surface of the cells thus interacting and activating CD4 T cells. On the other hand, antigens generated within the cells for example as a result of a viral infection, can be degraded by the proteosome and the peptides presented on the surface of the cell in the context of MHC I molecules[55,61,63]. This strategy selects and activates antigen specific CD8 T cells[55, 61,63]. Notably, DCs have the capability to cross-present antigens[64]. This means that DCs can acquire extracellular antigens, like for example apoptotic or necrotic tumor cells, or tumor lysates and also present them to CD8 T cells in the context of MHC I molecules.

DCS AND ANTI-TUMOR THERAPY

A multitude of preclinical studies and clinical trials have been designed in order to determine the anti-tumor efficacy and safety of DC-based vaccines[65]. The development of a successful DC-based tumor vaccination depends heavily on generating robust and long lasting specific CD4 and CD8 T cell responses[66]. To accomplish this, DCs have been generated from bone marrow precursors in the mouse and mostly from monocytes in humans as we previously reviewed[1]. Different steps in the antigen presentation process have been evaluated such as antigen loading, DC maturation, and delivery route and dose scheme as we have recently reviewed[1]. One strategy for loading DCs with TAAs in the mouse model involves pulsing the cells with peptides derived from tumor antigens[67]. In addition, since TAAs are not well characterized for the majority of tumors, vaccines can be prepared with whole tumor antigens[68,69]. To this end, DCs have been loaded with whole tumor lysates[70], apoptotic or necrotic cells[71] alone or conjugated with TLR ligands[72], antigens coated with antibodies to target them to Fcγ receptors[73] or peptides encapsulated in biodegradable polymers[74]. We have showed that inducing the expression of danger signals in tumor cells by means of replication-deficient or replication-restricted virus appears also to be an efficient method to pulse DCs for vaccination purposes, probably by upregulating danger signals in the tumor cells[71]. Finally, other strategies such as fusing DCs with tumor cells have also been successfully pursued[75]. These fused cells express tumor antigen but had the machinery of the DCs to present these antigens to T cells.

This information regarding DC-based antitumor vaccines pulsing has been translated to the human, where clinical trials have involved, among others, DCs pulsed with peptides[76], whole tumor lysates[77], or fused with tumor cells[78-80]. Other strategies involved pulsing human DCs with apoptotic or necrotic cells[81-90]. As we have previously reviewed[91] controversy exists regarding whether necrotic or apoptotic cells are better for pulsing DCs for tumor vaccination purposes[90,92-94]. Nevertheless, inducing tumor cell death by exposure to ultraviolet-B radiation seems to provide a mixture of apoptotic and necrotic cells suitable for vaccination purposes DCs[95,96].

TAA AS GENETIC MATERIAL

Another vaccination strategy entails delivering TAAs as

the genetic material that encodes their synthesis. Thus, either DNA or RNA carrying the information to synthesize TAAs can be administered to laboratory animals in preclinical studies or to patients under clinical trials with the aim to induce local synthesis of TAAs. In contrast to delivery of TAAs as protein/peptide formulations, the recombinant antigens synthesized in the cytosol of the cells may enter the degradation process of intracellular molecules, yielding peptides that can be directly presented by MHC I molecules hence inducing a robust CD8 (cytotoxic) T cell immune response. To this end, numerous studies have been performed in order to determine the effectiveness of DNA vaccination in tumor settings[49,97,98]. The genetic material can be administered *in vivo* by using different techniques such gene gun, ultrasound, electroporation, cationic liposomes, and nanoparticles[99]. Alternatively, viral vectors can deliver DNA encoding for TAAs directly to the DCs. Viral vectors used to transduce human DCs[100] include recombinant adenoviruses[101-103], poxviruses[104], and retrovirus[100]. Lentiviruses have also been used to induce stable transduction of human hematopoietic stem cells or DCs[105,106]. These vectors have the advantage of infecting non-dividing cells, therefore being excellent tools to express different molecules in terminally differentiated DCs which have lost the capability to duplicate. Moreover, hematopoietic stem cells have been transduced with lentiviruses and then differentiated into antigen-expressing DCs[107]. The full scope of DNA vaccination has been extensively reviewed in the literature and will not be discussed here.

RNA VACCINES

An alternative approach for delivering TAAs as genetic material is the use of RNA for vaccinations. The advantage of RNA vaccination in comparison to DNA vaccination is that there is no danger of genome integration with the latent possibility of oncogene activation, and that there is no need to engineer expression vectors for delivery. On the contrary the expression of the antigens in the context of RNA delivery is transient, and then RNA is very labile as compared to DNA. Both DNA and RNA vaccines in addition to carrying TAAs have the potential to non-specifically stimulate the immune response upon recognition of CPG sequences by TLR9 (DNA) or by activation of TLR3 (RNA). RNA vaccination strategies involve naked RNA delivery or the pulsing of DCs with RNA molecules. Further, both whole tumor RNA or TAA specific RNA have been used as inducers of antitumor immunity.

VACCINES WITH NAKED RNA

Murine studies

Several murine studies describe the use of naked RNA for vaccination purposes. The naked RNA can be administered by injection or delivered intradermally through electroporation[108]. In order to decrease degradation, the RNA has been complexed with histidine-rich cationic polymers and histidylated cationic lipids. In this case, systemic injections of specific synthetic messenger(m) RNA encoding the human melanoma MART-1 TAA complexed with polyethylene glycol ylated histidine-rich polylysine and histidylated liposomes (termed lipopolyplexes) were able to delay the growth of B16F10 melanoma in the mouse model[109]. Notably, intravenous injection of mannosylated liposomes containing mRNA encoding for the EGFP protein proved to be taken up by spleen DCs. Further, when mRNA for MART-1 was complexed into these mannosylated liposomes, a decrease in the growth of B6F10 murine melanoma tumors was observed[110].

Another strategy is to deliver naked RNA that could simultaneously activate the immune response by way of TLR signaling. These kind of vaccines are called "two component" since they deliver TAAs while simultaneously activating the immune response. It has been reported that two component OVA-encoding RNA vaccines containing free and protamine-complexed mRNA induced specific immune responses activating both humoral and cellular immune responses against OVA-expressing tumors[111]. In addition, naked RNA can be injected systemically, or can be administered directly to sites harboring high concentration of immune cells by means of intranodal injection[112,113]. This strategy aims to directly target APCs in the site where they interact with T cells.

An innovative approach to RNA vaccine immunotherapy has been the developing of self-replicating RNA vectors (replicons). These vectors encode for a RNA-dependent RNA polymerase derived from alphaviruses which has the capability to amplify a plasmid-encoded TAA RNA[114]. This increases the availability of TAA RNA and consequently, TAA protein availability. In addition, this counteracts the high degradation that naked RNA is subjected to upon injection. Immunization with RNA replicons encoding for HPV antigens was able to decrease the growth of aggressive TC1 tumors, which carry HPV E6 and E7 antigens[115].

Human studies

Naked RNA vaccinations have been assayed in clinical settings. In particular, naked RNA encoding for several TAAs has been delivered intradermally inducing expression of cytotoxic T cells in cancer patients, together with an improve on the clinical response in some individuals[116,117]. In order to enhance the effectiveness of the transfection process while protecting the RNA from degradation, naked RNA has also been delivered complexed with liposomes in human clinical studies[118]. Further, both in mouse and human studies, adjuvants that target APCs such as FLT3 and GM-CSF have been co-delivered in their protein state or as RNA together with the naked RNA vaccines in order to further activate these cells locally[118]. This strategy aims to induce a robust activation of the transfected DCs *in vivo*, thus potentiating their migratory potential and their ability to induce the activation of T cells capable of recognizing TAAs of interest.

USE OF RNA-PULSED DCS FOR ANTITUMOR THERAPIES

Mouse studies

Foundational studies evaluating the effectiveness of DC-based RNA vaccination in the mouse model and in humans were performed by Dr Eli Gilboa. In 1996 his group was able to demonstrate that murine DCs pulsed with whole tumor RNA were able to induce a robust antitumor immune response in a mouse model of melanoma[119]. Shortly after, they were able to demonstrate the feasibility of this approach in a preclinical setting, inducing specific T cell responses *in vitro* by pulsing human monocyte-derived DCs with the carcinoembryonic antigen (CEA) antigen[120]. Since then, a multitude of studies have built on these successes in order to generate efficient DC-based RNA vaccines.

In animal experimental models, the efficacy of RNA-pulsed DC vaccination has been extensively tested. Collectively, vaccinated animals showed a decrease in tumor growth together with the activation of tumor specific cell-mediated immunity. In particular, murine DCs have been pulsed with whole tumor RNA as a source of TAAs[121-125]. Interestingly, we have previously reported that DCs pulsed with whole tumor RNA are more effective in inducing antitumor immune responses than DCs loaded with equivalent amounts of apoptotic tumor cells[126]. In order to enhance antigen presentation by DCs and the consequent efficacy of the vaccination procedure, DCs have also been pulsed with specific TAA mRNA replicons[127]. As described above, these constructs aim to increase the amount of TAA RNA present in the APCs with the consequent increase in the levels of expression of the antigen.

Other strategies designed to increase the effectiveness of DC-based RNA vaccination entailed pulsing DCs with TAA mRNA together with mRNA of cytokines such as GM-CSF and particularly IL-12[128-131], the rationale being that these cytokines will potentiate the degree of activation of the pulsed DCs.

Alternative strategies focused on enhancing the processing of the nascent TAA in the transfected DCs. To this end, studies pulsing DCs with RNA encoding for TAAs fused with molecules that augment the delivery of the synthesized proteins to the endoplasmic reticulum, TAAs RNA linked with ubiquitin RNA to target the ubiquitin-proteosome pathway, MHC I and Ⅱ pathways by fusion with LAMP1 or DC. LAMP sequences, or with immunogenic helper proteins such as EGFP have been used[97,132-134]. In this way, cytoplasmic TAAs will be more efficiently processed by the ER, increasing the levels of TAAs peptides presented in the context of MHC I molecules on the surface of the DCs.

Finally, others strategies to potentiate the efficacy of DC-based RNA vaccines entail the use of different maturation cocktails or immunostimulatory factors to activate the RNA-pulsed cells. For example, soluble CD40 has been shown to act as an adjuvant for cytokine treatment of RNA-pulsed DCs increasing the generation of cytotoxic T cells in a an experimental model of melanoma[135].

Human preclinical

In order to optimize the likelihood of effective translation into the clinic, human DCs have been prepared from monocytes recovered from apheresis products or by differentiation of $CD34^+$ hematopoietic precursors[136,137]. As above, whole tumor RNA or mRNA can be used to transfect these cells by electroporation or lipofection[137,138]. In addition, RNA recovered from tumor cells lines can be used to pulse human DCs. For example, whole RNA from KL562 leukemia cells was delivered to monocyte-derived DCs by electroporation and lipofection being the transfected RNA degraded within 24 h. Notably, the translated TAA proved to be processed through the MHC-I presentation pathway rather than the endosomal-phagocytic pathway indicating that these DCs could be able to activate CD8 cytotoxic T cells[139]. Interestingly, not only monocyte or hematopoietic $CD34^+$ derived DCs have been tested in RNA vaccination studies. Indeed, DCs directly recovered from hepatocellular carcinoma patients could be efficiently pulsed with whole RNA recovered from hepatic cancer cell lines[140].

It has been determined that better expression of TAAs after transfection with whole tumor RNA is achieved when antisense RNAs are eliminated from the whole tumor RNA preparation[141]. This highlights the need to prepare high quality RNA for transfection studies. Further, although most of DC protocols (both in mouse and human) propose to induce maturation of these cells after RNA transfection, a study suggests that RNA transfection of DCs can also be performed after maturation of these cells[142]. Taking into account studies indicating the viability of cryopreserved mature human DCs[143], this opens the possibility of transfecting DCs right before administration to patients.

Human DCs transfected with tumor RNA have been shown to elicit specific T cell responses *in vitro*. This was demonstrated by their ability to generate TAA specific T cell lines, or by activating *ex vivo* TILs recovered from cancer tissues. For example, DCs transfected with survivin or TERT RNA were used to generate CD8 cytotoxic cell lines with the capability to eliminate tumor cell lines and primary tumors *in vitro*[144,145]. Further, RNA recovered from prostate tumor samples by laser capture microdissection was amplified and used to transfect DCs generated from blood precursors. It was shown that these DCs were able to induce cytotoxic T cells *in vitro*[146].

As described above, mouse studies determined that RNA encoding for TAAs can be engineered to enhance the capability of the DCs to process the nascent antigens. To translate these results into the human setting, DCs generated from human monocytes were transfected with mRNA encoding for the TERT antigen fused with LAMP in order to augment the processing of the TAA upon translation. This strategy induced a robust activation of CD4 T cells specific for TERT as determined in

in vitro studies[147].

Human clinical studies

Several clinical trials have been conducted in order to evaluate the efficacy of DC-based RNA vaccines in cancer patients. In these clinical trials, the vaccines were generated by pulsing monocyte-derived DCs either with whole tumor RNA or specific TAA RNA. Altogether, human clinical studies highlight that the administration of DC-based RNA vaccines is safe and does not induce adverse reactions. For example, in a phase I clinical trial involving acute myeloid patients aiming to generate clinical grade DC vaccines, monocyte-derived DCs were pulsed with *in vitro* transcribed RNA encoding the Wilm's tumor. Then, these cells were injected repeatedly into patients by the intramuscular route[148]. The results of this study indicated that the vaccination scheme was well tolerated by the patients[148]. This was also observed in a clinical trial involving stage IV malignant melanoma patient[149]. In this case, DCs were pulsed with whole tumor RNA expanded *in vitro* but no positive effect of the vaccination was observed. This is no surprising taking into account the advanced stage of the illness, but nevertheless the study highlights the safety of using this procedure for antitumor therapies. Other studies showed that DC-based RNA vaccination is able to induce specific T cell responses in cancer patients. In particular, in a clinical trial involving relapsed metastatic ovarian cancer patients, DCs pulsed with mRNA specific for folate receptor α were able to induce a large population of effector memory CD8 cytolytic T cells reactive to the antigen upon repeated injections[150]. Similarly, specific T cell responses were observed in colorectal cancer patients receiving several injections of DCs harboring CEA mRNA[151]. In addition, it has been shown that patients vaccinated with DCs transfected with mRNA recovered from autologous melanoma tumor cells were capable of initiating T cell responses specific to antigens encoded by the pulsed APCs[152]. Finally, in order to ensure a robust activation of T cells, strategies designed to deliver the transfected DCs directly to the lymph nodes have been tested. In a phase Ⅰ/Ⅱ clinical trial with melanoma patients it has been shown that upon intranodal administration, DCs electroporated with mRNA encoding for gp100 or tyrosinase migrate towards T cells areas of the lymph node [153].

CONCLUSION

In closing, in the last 15 years, a growing body of literature has argued for the use of RNA for vaccination purposes. Importantly, RNA is safer than DNA vaccine approaches taking into account that no possibility of genomic integration exists. Furthermore, the combination of RNA technology with DC-based vaccines has made available a powerful strategy for antitumor therapies. Advances in RNA technology (*i.e.*, strategies to increase stability, use of replicons), together with the development of more effective protocols for generating activated DCs (*i.e.*, use of better inflammatory cocktails) and an increase in our knowledge of tumor immunology (*i.e.*, the use of immunoablative therapies to eliminate suppressor populations) will guide further pursuit of tumor immunotherapies using DC-based RNA vaccines. This offers the potential to advance the outcome of cancer immunotherapies for the benefit of patients.

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