Chapter 4

Vaccines Developed for Cancer Immunotherapy

Aizong Shen, Rui Qian, Ting Liu, Qingchuan Liu,
Bin Liu and Zhangbao Wu

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.80889

Abstract

Vaccines have been successfully used for prophylaxis of infectious diseases for a long time and in the last decades have inspired researchers to make products with similar immunological mechanisms for cancer immunotherapy, which has been developed rapidly into clinical applications and has shown remarkable therapeutic efficacy, as exemplified by chimeric Ag receptor T cell (CAR-T cell) and immune checkpoint inhibitor-based therapies which can efficiently strengthen the body's immune system to fight against cancer, but they are also expensive. Therefore, encouraged by recent success of cancer immunotherapy, scientists are actively developing the low-cost tumor Ag-based vaccines, which, however, usually exhibit weak immunostimulating effects and, therefore, are often formulated with nanoparticulate carriers to form a vaccine adjuvant-delivery system (VADS), which can not only enhance the efficacy but also mitigate the off-target toxicity associated with conventional anticancer vaccines. These nanoparticulate carrier-based VADSs have demonstrated multiple functions, such as targetedly triggering Ag-presenting cells, reeducating tumor-associated macrophages (TAM) to function as tumor suppressor agent, and eliciting robust cytotoxic T lymphocytes (CTLs) to kill tumor cells. This chapter introduces multifunctional VADS that have been engineered with nanoparticulate carriers, including polymeric-, lipid-, metallic-, and cell-based nanoparticles, and used as an alternative to the existent tools for cancer immunotherapy.

Keywords: cancer immunotherapy, vaccine adjuvant-delivery system (VADS), immunoresponse, nanocarrier, cellular immunity, immune surveillance, danger-associated molecular pattern

1. Introduction

The huge success in vaccination against infectious diseases inspired researchers to explore the principles of immunotherapy for controlling tumor growth using host immune system,

which has been tried to be triggered with a variety of strategies, such as immune checkpoint inhibitor- and engineered T cell-based therapies, to elicit antitumor immunity in the body and has shown great potential in treatment and prevention of recurrence of cancer, as exampled by recent striking outcomes of cancer immunotherapy in clinical applications [1, 2]. From the overall view, current cancer immunotherapy is usually undertaken in two ways: establishment of systemic immunity through utilizing cytokines, vaccines, or adoptive cell transfer (ACT) and regulation of local immunosuppressive tumor microenvironment through utilizing small molecules and immune checkpoint inhibitors. Immune checkpoint therapy addresses regulatory pathways in preexisting Ag-specific T cells aiming at enhancing antitumor immune responses, whereas self-sustaining systemic anticancer immunity proceeds with anticancer immune response, which is dictated by both vaccines and TME [3]. However, immune checkpoint inhibitors are mostly aimed at augmenting the potency of preexisting tumor-specific T cells and as such benefit only a portion of patients [2], while the strategies based on the engineered T cells involve complex bioengineering processes with almost an unacceptable cost and, sometimes, off-target severe toxicities [4]. These situations compel researchers to develop other anticancer tools, including, especially, the tumor Ag-containing vaccines, which trigger the immune system to establish anticancer immunity through several crucial processes: release of Ags from tumor beds to be taken up by Ag-presenting cells (APCs) or delivery of Ags to APCs, APC activation for presentation of tumor Ags, priming and activation of T cells by activated APCs, migration and infiltration of effector T cells back to the tumor, and finally the recognition and killing of tumor cells by effector T cells, each of which, theoretically, can be targeted with various therapeutic approaches [5]. In particular, cancer vaccines that are designed for targeting early steps of Ag processing are potentially able to enhance both therapeutic and prophylactic efficacies against not only primary tumor but also inoperable metastasis or relapse and will therefore benefit a wide range of patients, especially the ones that lack sufficient levels of preexisting tumor-specific T cells and immune checkpoint-related molecules [6].

However, despite having in expectation tremendous therapeutic potential, cancer vaccines designed in conventional ways have been found elusive for successful treatment and eradication of tumors due to their weak immunogen insufficient to induce immune responses with conventional vaccination approaches. In addition, there are several other issues that may block the establishment of anticancer immunity, including degradation and rapid elimination of Ag, ineffective DCs uptake and Ag presentation, the suppression of T cell functions, and impairment of Ag presentation by the endoplasmic reticulum (ER) stress-driven lipid metabolism in DCs, thereby inhibiting protective T cell responses in cancer immunotherapy [7]. All of these highlight the need for developing new strategies to prepare cancer vaccines that can efficiently deliver tumor Ags and adjuvants to APCs and stimulate immune responses strong enough to kill tumor cells [8]. In this regard, NPs, such as liposomes, polymeric aggregates, and inorganic NPs, when used as a vaccine carrier have proven to be able to enhance the accumulation in draining lymph nodes (dLNs) of immunostimulants and adjuvant Ags, which thereby approach and stimulate a large number of Ag-presenting cells (APCs) enriched in dLNs to initiate cellular immunity required for fighting against cancer [6]. Moreover, several decades of intensive investigation on different types of NPs for targeting delivery

of traditional chemotherapeutics to solid tumors provide basis of repurposing these NPs to target the immune system and offer new opportunities to tune immunity and elicit strong antitumoral immune responses for overcoming the major challenge to the clinical translation of cancer vaccines [9].

Actually, multifunctional NPs show numerous advantages over conventional therapeutics for cancer immunotherapy: (1) NPs with finely tuned size and a defined surface features can achieve targeting delivery to lymphoid tissues, while most NPs composed of biomaterials bearing immune-stimulating properties may serve a dual role as a vaccine carrier and an adjuvant, thus forming a vaccine adjuvant-delivery system (VADS) to simplify the vaccine production [10–12]; (2) NPs carrying both tumor Ags and adjuvants can stably co-deliver vaccine components to APCs [13, 14]; (3) NPs can also be to display Ags and co-stimulatory ligands to serve as artificial APC and potentiate T cell immune responses [15]; (4) VADSs can be formulated to trigger immunogenic cell death or target immune checkpoint molecules leading to antitumoral immune responses and reverse of immune suppression [16]; and (5) VADS can also be loaded with therapeutics to improve antitumoral efficacy of adoptive T cell therapy [17].

This chapter elaborates new developments in areas of cancer immunotherapy, highlighting the potential of the VADSs engineered with various types of NPs for developing vaccines that are explored for cancer immunotherapy.

2. Cancer immunity and immunosuppression

Vertebrates are protected by the immune system from pathogens such as viruses, bacteria, fungi, and parasites through immune responses which can be classified into two categories, namely, innate and adaptive processes thus to establish, respectively, two types of immunity: the innate immunity providing rapid defense against pathogens and the more comprehensive adaptive immunity, which is set up requiring process of pathogens by professional APCs for presentation of immunogenic Ags to T and B cells to sponsoring cellular and humoral immune responses. Professional APCs, including B cells and macrophages, as well as dendritic cells (DCs) which are considered as the most efficient APC population, have proven to play a pivotal role at the interface of innate and adaptive immune responses [18, 19]. To initiate immune responses, DCs take up and then process endogenous or exogenous Ags in the context of major histocompatibility complex (MHC) class I or II, followed by presentation of the MHC-I or -II/Ag peptide complex as the activation "signal 1", respectively, to CD8+ and CD4+ T cells, which are activated requiring an additional "signal 2" induced by ligation of co-stimulatory markers CD80/86 on DCs with CD28 on T cells, as well as a T cell polarizing "signal 3" provided by cytokines, such as interleukins (ILs) and interferons (IFNs) secreted by DCs [6]. Although MHC-I is constitutively expressed by most mammalian cells, nonprofessional APCs can never present "signals 2 and 3" to alert the immune system after invasion by pathogens, highlighting the Ag processing and presentation by APCs as a critical first step in sponsoring adaptive immune responses. In particular, DCs rather than other APCs prove to be able to process exogenous pathogens to activate CD8+ T cells via a unique process called cross-presentation, of which, though the exact mechanisms are still unclear, the vacuolar and

cytosolic pathways have been identified to utilize endosomes and endoplasmic reticulum, respectively, to generate the MHC-I/Ag peptide complexes [20–22]. Notably, the endosome pathway in normal conditions is devoted to process the exogenous Ags to form the MHC-II/Ag peptide complexes for activation of CD4+ T cells, although the abnormal increase in endosomal pH or occurrence of endosome breakup made purposely by artificial strategies is thought to prevent the protease-mediated degradation of Ags in endosomes, thus promoting cross-presentation [12, 23], and additionally, certain DC subsets such as tissue-resident CD8+ and migratory CD103+ DCs in mice and CD141+/BDCA-3+ DCs in humans were reported to be more efficient at Ag cross-presentation than other DC subtypes [24, 25].

After activation and differentiation from CD8+ T cells in lymphoid tissues, the matured Ag-specific cytotoxic CD8+ T lymphocytes (CTLs) enter the systemic circulation and patrol peripheral tissues in search of target cells, which display a specific Ag epitope in the context of MHC-I matching the Ag-specific T cell receptors (TCRs) on CTLs, which once identification of the target cells will secrete perforin and granzymes to lyse them and within minutes move on to kill the next target [26]. By contrast, CD4+ T cells mainly play a helper role of regulation of immune responses as manifested by the observations that after activation by MHC-II/Ag peptide complex presented by DCs, naïve CD4+ T cells differentiate into four distinctive subtypes depending on the polarizing cytokines [27]. Type 1 helper T cells (Th1) induced by IL-12 secrete IL-2 and IFN-γ to promote CD8+ T cell responses; Th2 cells induced by IL-4 secrete IL-4 and IL-5 and are involved in humoral immune responses; regulatory T cells (Tregs) induced by IL-2 and TGF-β (transforming growth factor beta) secrete TGF-β and IL-10 to suppress immune responses; and Th17 cells induced by TGF-β, IL-6, and IL-21 secrete IL-17 and IL-22 to break immune tolerance and possibly leading to autoimmunity [27, 28]. In addition, it is reported that CD4+ helper T cells are utilizing the expressed CD40L for feeding back to DCs to further amplify immune activation and aid in establishment of memory CD8+ T cell responses [29, 30].

To prevent cancerous occurrence, the immune system constantly implements a process referred to as immunosurveillance whereby to inhibit oncogenesis by actively identifying and eliminating tumor cells, which however, have also devised mechanisms to evade immune responses, including downregulation of tumor Ags and promotion of immunosuppression [31, 32]. In established tumor microenvironment, it is generally immunosuppressive due to upregulation or production of inhibitory molecules, such as TGF-β1, CXCL12, VEGF, ARG1 (Arginase1), CCL18, iNOS (nitric oxide synthase), IL-10, IL-35, and galectin-1 by many types of cells, including cancer-associated fibroblasts, myeloid-derived suppressor cells, Tregs, and tumor-associated macrophages (TAMs), against T cells [33]. Also, activated T cells upregulate CTLA-4 (CTL-associated protein 4) which binds to co-stimulatory molecules on DCs with higher affinity than CD28, serves as a peripheral inhibitory signal to prevent over-reactivity of T cells, and dampens antitumor immune responses. Besides, tumor cells can also secrete cytokines such as IL-10 and TGF-β, which both directly inhibit the proliferation of CTLs and drive the differentiation of Tregs to provide an additional source of immunosuppressive cytokines, while subsets of tumor cells highly express programmed death-ligand 1 (PD-L1) for binding to programmed death-1 (PD-1) on T cells and inhibiting their effector functions [34]. Thus, tumor cells can promote immunosuppressive tumor microenvironment and shield themselves from CTLs by hijacking normal negative feedback loops designed to guard against

excessive activation of T cell responses, suggesting that development of vaccines for cancer immonotherapy is still confronting a huge challenge arising from cancer immunosuppression.

3. NP entrapping various Ags for delivering cancer vaccines

Tumor Ags include mutated cell surface components, such as polysaccharides, peptides, oncoproteins, and DNA and mRNA that encode those proteins, which as referred to subunit Ags, meanwhile tumor cell lysate and immunogenically dying tumor cells can also serve as the source of whole-cell Ags [6]. As key components utilized for formulating anticancer vaccines, subunit Ags have major advantages including defined chemical synthesis; ease of production; and for vaccine formulations, requiring, possibly, no Ag-processing by APCs and challenges including elicitation of humoral rather than cellular immune responses, poor delivery efficiency, and in vivo stability. Whole-cell Ags have major advantages including broad-epitope immune responses, potential for "personalized" therapy, full preservation of tumor Ags and challenges including production requiring tissue biopsy, difficulty in manufacturing, loss of antigenicity during production, presence of self-Ags, and immunosuppressive molecules such as PD-L1. Notably some viruses, such as Epstein–Barr virus (EBV), human papilloma virus (HPV), and hepatitis B and C viruses, have proven to contribute to certain cancer-related development, and therefore, their virally gene encoded surface proteins may also serve as the potential target Ags to constitute the vaccines for cancer immunotherapy [35, 36]. Among different types of tumor Ags, oncoproteins, which are encoded by oncogenes involved in the regulation or synthesis of proteins linked to tumor cell growth and may also be either mutated or overexpressed normal or embryonic proteins from fetal development, are intensively investigated for cancer vaccines since they have a big potential in induction of broad-epitope CD8+ and CD4+ T cell responses. Notably, compared to full-length protein-based Ags that require cellular uptake and processing for presentation to T cells, peptide epitopes can directly bind to MHC molecules and thus directly activate T cells and, moreover, are more endurable to damages during the preparation and storage of vaccine products, thus, in line with these advantages, leading to many ongoing clinical trials on peptide-based cancer vaccines [37, 38].

However, poor immunogenicity and limited therapeutic efficacy are still big challenges in developing protein, especially, peptide Ag-based subunit vaccines that are designed for cancer immunotherapy; for example, in the case of melanoma, the identified Ags include β-catenin, survivin, tyrosinase, gp100, MAGE, melan-A (MART1), and NY-ESO-1, some of which, such as gp100 and MAGE-A3 peptides, when tested in clinical trials just showed only moderate or null therapeutic efficacy [39]. Grooming through clinical trials on peptide-based cancer vaccines, it may be safely concluded that therapeutic efficacy of subunit vaccines against cancer remains suboptimal [2], due to at least partially the fact that many tumor Ags evaluated in clinical trials are self-Ags which can hardly trigger the autoreactive T cells leading to immunotolerance [6]. These disappointed outcomes highlight that the conventional subunit vaccines should actually be formulated with innovative modalities, which may be an alternative promising strategy to further improve cancer immunotherapy, as evidenced by positive results obtained from pre- and clinical investigations carried out more recently

on cancer vaccines that were combined with other elements, such as potent adjuvants and NP-based VADSs. For example, in a preclinical study, researchers observed that, in a syngeneic mouse model of oral cancer comprised of mouse tonsil-derived epithelial cells stably expressing HPV-16 E6 and E7 genes along with H-ras oncogene (mEER), intranasal HPV E6/E7 peptide vaccination or single checkpoint antibodies failed to elicit responses in most mice; however, 4-1BB agonist antibody along with either CD40 agonist antibody or CTLA-4 blockade eliminated the majority of established mEER tumors, and even produced a curative efficacy and a high safety profile against orally implanted mEER tumors [40]. For another example, in a phase II clinical trial, researchers performed immunotherapy with two peptide cancer vaccines in combination with intravesical bacillus Calmette-Guerin (BCG) for patients with non-muscle invasive bladder cancer (NMIBC) and demonstrated that this combinatory immunotherapy had good immunogenicity and safety and resulted in a 2 year RFS rate 74.0% in all patients, suggesting the cancer vaccines with a combinatory mode may provide benefit to patients for preventing recurrence of NMIBC [41].

These investigations showed that the conventional vaccines have limited capability to target delivery of tumor Ags and adjuvants to proper APC and intracellular compartments and may be renovated by the NP-based vaccine adjuvant-delivery systems (VADSs) which have already poised to address these challenges as described below.

4. NPs delivery of cancer vaccines

Cancer immunotherapy by vaccines depends on eliciting in patient the antitumor adaptive cellular immunity, which is, however, governed by potent Ag-presenting DCs able to activate CD8+ T cells and engender the Ag-specific CTLs. For this purpose, various immunotherapy strategies have been developed, including, in particular, using NP-based VADSs that are so elaborately designed as to promote APC cross-presentation of Ags and to deliver Ags and/or adjuvants targeting APCs, tissues, or organs such as dLNs, wherein APCs aggregate in large number ready for uptake of foreign substances, as can hardly be accomplished by soluble Ags or adjuvants alone [12].

4.1. NPs promoting Ag cross-presentation for delivering cancer vaccines

During an immune response, exogenous Ags are usually processed and presented via MHC-II by APCs to CD4+ T cells; however, tumor Ags engulfed by APCs require to be presented via MHC-I to induce production of Ag-specific CTLs, which are the main effector cells against tumor cells, thus precluding traditional methods from engineering cancer vaccines as they rely on soluble protein or peptide tumor Ags which often skew immune responses to CD4+ T cell responses while failing to induce robust CTL responses which are sufficient for cancer immunotherapy. Fortunately, it is disclosed that tumor Ags delivered by the elaborately designed NP-based multifunctional VADS able to promote lysosome escape, which is translocation of Ags from endosomes or phagosomes to cytosol avoiding Ag degradation within lysosomes, may regulate Ags to be reloaded to endoplasmic reticulum (ER)-attached MHC-I

for cross-presentation and favorably elicit CD8+ T cell responses [24]. As such, to engender Ag lysosome escape, great efforts have been focused on pH-sensitive delivery systems that can retain the loaded cargo under the physiological pH condition while triggering release of Ags and disruption of endocytic vacuoles at the acidic (below pH 6) endosomal microenvironment [42], as exemplified by a pH-sensitive liposomal VADS which is formulated with a dextran derivative and was shown to promote cytosolic delivery of Ags [43].

More recently, Wang and colleagues through fabricating two types of pH-sensitive multifunctional liposomes, the mannosylated lipid A-liposomes (MLLs) and the stealth lipid A-liposomes (SLLs) both loaded with Ags and NH_4HCO_3, into microneedles prepared the proSLL/MLL-constituted microneedle array (proSMMA), which dissolved rapidly recovering the initial MLLs and SLLs upon rehydration [12]. Mice vaccinated with proSMMAs by vaginal mucosa patching elicited robust Ag-specific humoral as well as cellular immunity at both systemic and mucosal levels, especially, in the reproductive and intestinal ducts. Further exploration revealed that the Ags delivered by either liposomes were cross-presented for MHC-I displaying by APCs thanks to lysosome escape and reactive oxygen species stimulation, both of which occurred when lysosomal acidifying the liposome-released NH_4HCO_3 into CO_2 and NH_4^+/NH_3 to rupture lysosomes by gas expansion and to cause ROS production by excessive ammonia induction, resulting in a mixed Th1/Th2 type response which was also promoted by liposomal lipid A via activation of TLR4, indicating the proSMMAs a multifunctional VADS capable of engendering Ag lysosome escape to elicit robust humoral and cellular immunity against Ags and a promising platform for making both cancer and infection vaccines.

In addition, an alternative approach for evading lysosome degradation of Ag includes multifunctional VADS constituting of the oxidation-sensitive polymersomes that can respond to the oxidative environment of endosomes and deliver Ags and adjuvants to cellular cytosol for induction of cellular immune responses [44]. Notably, liposomes modified with a cell-penetrating peptide octaarginine were also reported to be able to promote cross-presentation Ags and elicit production of anticancer CTLs, because the membrane-penetrating liposome enhanced proteolysis of the exogenous Ags by proteasomes and amino peptidases facilitating promoting the C-terminal trimming of antigen peptide and the production of mature MHC-I peptides [45]. Also, gold nanoparticles displaying tumor Ags were reported to enable efficient antigen delivery to dendritic cells and then activate the cells to facilitate cross-presentation, inducing Ag-specific CTL responses for effective cancer immunotherapy [46].

4.2. NPs targeting DC for delivering cancer vaccines

Recently, the approach based on amphiphilic polymer-Ag peptide conjugates through the conjugation of azide-functionalized Ag peptides to an alkyne-functionalized core via azide-alkyne click chemistry has been employed for making nanovaccines against cancer. For example, by conjugation of the melanoma Ag peptide TRP2 and azido PEG mannose to the alkyne polymer, an anti-melanoma nanovaccine with the size of 10–30 nm was formed via self-assembly and was efficiently taken up by DCs [47]. In spite of poor immunogenicity, when given to model mice with B16-F10 melanoma tumors together with the adjuvant CpG, the adjuvanted TRP2-nanovaccines effectively suppressed the tumor growth and significantly

improved the survival of mice compared to the untreated group. Moon's group engineered synthetic high density lipoprotein (sHDL) nanodiscs consisting of phospholipids, apolipoprotein A1 (Apo A1)-mimetic peptides and cholesterol-conjugated CpG (sHDL-Ag/CpG) with average diameter of 10 ± 0.5 nm, which were used as a multifunctional VADS able to target lymphoid organs, resulting in sustained Ag-presenting on DCs [48]. Moreover, the sHDL-CpG-based VADS loaded with multiple Ags (MHC-I-restricted M27, MHC-II-restricted M30, and TRP2) in combination with anti-PD1/anti-CTLA4 antibodies successfully rejected B16-F10 tumor from tumor-bearing mice.

Though targeting delivery with NPs is able to improve efficacy of cancer vaccines, tumor-induced DC dysfunction arising from hyperactivity of signal transducer and activator of transcription 3 (STAT3) [49], which leads to less maturation in DCs with low responsiveness to pattern recognition receptor agonist (PRRa) stimulation [50], engenders another major huddle to developing effective vaccines for cancer immunotherapy. The NPs-based VADS was trialed in overcoming tumor-induced DCs dysfunction by Ma and colleagues through using poly(ethylene glycol)-b-poly(L-lysine)-b-poly(L-leucine) (PEG-PLL-PLLeu) to form 120 nm-sized polypeptide micelles for encapsulation of polyI:C, STAT3 siRNA, and OVA as a nanovaccine (PMP/OVA/siRNA), which proved able to decrease STAT3 expression and increase CD86 and CD40 expression as well as IL-12 production [51]. Moreover, PMP/OVA/siRNA nanovaccine could effectively increase mature DCs and decrease immunosuppressive cells in tumor draining lymph node, leading to antitumor immune response and prolonged survival, implying that novel VADSs designed for co-delivery of immunopotentiator and immunosuppressive gene silencer may be one of potent strategies to improve antitumor immunity by modulating tumor-induced DCs in tumor microenvironment.

4.3. NPs targeting the lymph node for delivering cancer vaccines

Pattern recognition receptors (PRRs) are germline-encoded host sensors expressed mainly by cells of the innate immune system, such as MPs, (MPs) macrophages, monocytes, neutrophils, and epithelial cells, capable of detecting two classes of molecules: pathogen-associated molecular patterns (PAMPs), which are associated with microbial pathogens, and damage-associated molecular patterns (DAMPs), which are associated with components of host's cells that are released during cell damage or death [52]. PRRs play a crucial role in the proper function of the innate immune system evolved before other parts of the immune system, particularly before adaptive immunity, and mediate the initiation of Ag-specific adaptive immune response and release of inflammatory cytokines when they are activated by PRRas (PRR agonists), which are the microbe-specific molecules, including bacterial carbohydrates such as lipopolysaccharide (LPS) and mannose, bacterial peptides such as flagellin and microtubule elongation factors, peptidoglycans and lipoteichoic acids, nucleic acids such as bacterial or viral DNA and RNA, fungal glucans, and also chitin and thus are often used as vaccine adjuvants.

Since a fraction of PRRas such as LPS and unmethylated CpG ODN are soluble, prevention of rapid diffusion of free PRRas into the systemic blood circulation is indispensable

for efficient targeting to professional APCs, which may be well obtained through formulating into the NP-based VADSs. This has been accomplished by dextran-CpG-OVA conjugate that enhanced not only the CD8+ T cell responses but also improved the antitumor immunotherapy through whole tumor cell vaccine. Recently, Liu and coworkers using reductive amination method conjugated oxidized dextran to amine-modified CpG ODN and demonstrated that the dextran-CpG conjugate with a hydrodynamic diameter of 6.5 nm was accumulated dLNs and was efficiently taken up by mouse DCs [53]. With the combination of OVA as a model Ag, dextran-CpG conjugate elicited production of Ag-specific CD8+ T cells for effective therapeutic benefits and in subcutaneously immunized mice resulted in significant reduction of tumor growth and increased survival of mice.

To induce a potent MHC-I-restricted CTL response which is an essential component of the successful cancer immunotherapy treatment, Huang's group formulated the mannosylated lipid-calcium-phosphate (MLCP) NPs as a new class of intracellular delivery systems for cytosol delivery into DCs of an exogenous Ag, p-Trp2 (the melanoma Ag Trp2 peptide derivative bearing two phosphor-serine residues) [54]. Compared with free Trp2 peptide/CpG ODN, MLCP NPs encapsulation enhanced and prolonged the cargo deposit into the lymph nodes (LNs) and also resulted in superior inhibition of tumor growth in both B16F10 subcutaneous and lung metastasis mouse models owing to induced IFN-γ production and a Trp2-specific CTL immune response. Thus, encapsulation of phospho-peptide Ags into LCP may be a promising strategy for enhancing the immunogenicity of poorly immunogenic self-Ags for cancer therapy.

Recently, a nanovaccine, called AlbiVax that is assembled in vivo from endogenous albumin nanocarriers and exogenous molecular vaccines, which are chemically defined and relatively well suited to large-scale production including quality control and safety evaluation, has been developed based on the albumin properties which are well known of not only being efficiently internalized by APCs via endocytosis to facilitate intracellular vaccine delivery for optimal Ag processing and presentation but also binding to a clinically practiced Evans blue (EB) [55]. AlbiVax was synthesized by conjugating thiol-modified vaccines and adjuvants, such as the 3′-end thiol-modified CpG and Ags (CSIINFEKL, Trp2, and Adpgk) modified with N-terminal cysteine, with maleimide-functionalized EB derivative which can tightly bind to human serum albumin. Further investigation revealed that, compared to benchmark incomplete Freund's adjuvant (IFA), AlbiVax had a much high efficiency in co-delivery of CpG and Ags to LNs and in eliciting peripheral Ag-specific CD8+ CTLs with immune memory and specifically inhibited progression of established primary or metastatic EG7.OVA, B16F10, and MC38 tumors; but only in combination with anti-PD-1 and/or Abraxane did AlbiVax eradicate most MC38 tumors. These outcomes indicate that as a novel type of VADS, the in vivo self-assembled molecular nanovaccines can not only enhance vaccine bioavailability in LNs but also bypass the complications, such as inefficient delivery, sequestering Ag determinant-specific T cells in the depots, and exhausting and depleting T cells, thereby preventing T cells from infiltrating tumors and difficulty in large-scale production, which are often associated with conventional synthetic vaccines [56].

4.4. NPs in combination for delivering cancer vaccines

The VADs based on various NPs that are engineered with different adjuvants such as PRRas and tumor Ags in combination for delivery of vaccines to the same immune cells have a great potential in provoking immune responses, generating increased duration and speed of immune response, regulating Ag-antibody response, and amplifying immunogenicity of weak Ags [12]. For example, it is reported that the application of poly(γ-glutamic acid)-based NPs for the delivery of model Ag (OVA) and toll-like receptor 3 (TLR3) agonist poly (I:C) (polyinosinic-polycytidylic acid) in targeting the LNs significantly enhanced the antitumor immunity against EG7-OVA (EL-4 thymoma cells transfected with chicken albumin cDNA) in tumor-bearing mice [57]. Recently, Molino et al. designed a biomimetic approach for eliciting antitumor responses through engineering the viral-mimicking protein NP vaccine, which is pyruvate dehydrogenase E2 protein NP (of 50 nm) conjugated to gp100 epitope (melanoma-associated Ag) and CpG [58]. The CpG-gp-E2 NPs remarkably increased the proliferation of Ag-specific CD8+ T cells and production of IFN-γ and dramatically enhanced the population of CD8+ T cells in dLNs, resulting in the delayed onset of tumor growth in mice as well as elevated mouse survival, compared to control PBS-treated animals.

It should be pointed out that delivery of vaccine Ags and adjuvants to target tissues or cells by a VADS is also, to a great extent, dictated by NP properties, such as particle size and surface charge, which may be appropriately engineered for improving their delivery efficiency [59]. For example, the NP-based vaccines could be either delivered actively to the lymph nodes by DCs in target tissues or transported by the interstitial flow into the lymphatics, depending mainly on NP size and surface properties such as PEGylation and charge due to the upper limit of pore size of the lymphatic capillaries and cell uptake of NPs relevant to surface properties of both [12, 60]. Wang's group engineered two types of multifunctional liposomes, the mannosylated lipid A-liposomes (MLLs) with a size of 200 nm and the stealth lipid A-liposomes (SLLs) of 50 nm, both of which were loaded with a model Ag and NH_4HCO_3 and fabricated into microneedles, forming the proSLL/MLL-constituted microneedle array (proSMMA) as a multifunctional VADS [12]. Mice vaccinated with proSMMAs by vaginal mucosa patching administration established robust Ag-specific humoral and cellular immunity at both systemic and mucosal levels, especially, in the reproductive and intestinal ducts, under the revealed mechanism that the MLLs reconstituted from the administered microneedles were mostly taken up by vaginal mucosa resident DCs, whereas the recovered SLLs trafficked directly to dLNs wherein they are to be picked up by macrophages, proving the size of NPs as an important parameter in controlling the in vivo fate of the delivered vaccines.

5. NPs for delivering DNA and mRNA vaccines

Using DNA and mRNA for intracellular production of oncogenic proteins or peptides as tumor Ags becomes an attractive strategy for developing cancer vaccines thanks to the advances in biotechnology which allows gene encoding proteins of interest to be easily manufactured in batch and be further modified with nucleic acid sequences that encode for proteins with immunostimulatory functions, for example, flagellin and a toll-like receptor

5 agonist (TLR5a). Unfortunately, previous clinical trials on DNA cancer vaccines, majority of which were administered as naked DNA via the intramuscular route, showed generally poor response rates, despite employment of viral vectors and electroporation able to improve the transfection of DNA vaccines, both of which cause safety and compliance concerns [61, 62]. Alternatively, NPs engineered as a VADS for intracellular delivery of DNA and mRNA provide a promising strategy for developing nucleotide-based cancer vaccines and possess several advantages [6]: (1) synthetic material-constituted NPs are safer than viral vectors, (2) NPs can stabilize and protect gene therapeutics from nuclease-mediated degradation [63], (3) DNA- and RNA-loaded NPs can be administered by injection-free tools, such as microneedles for non-parenteral delivery [64], and (4) nanocarriers can be easily modified with targeting moieties, for example, mannose, to achieve DC-targeted delivery and transfection [14, 65].

It is reported that cationic liposomes and lipid nanoparticles containing mRNA coding for the tumor-associated Ags gp100 and TRP2 could induce a strong CD8+ T cell activation after a single immunization and treatment of B16F10 melanoma tumors with the mRNA-carried cationic liposomes resulted in tumor shrinkage and extended the overall survival of the treated mice, all of which could be further increased by the combinatory incorporation of the adjuvant LPS, showing the cationic liposomes a promising vector for mRNA vaccine delivery that is capable of inducing a strong cytotoxic T cell response for cancer immunotherapy [66]. Nevertheless, nucleotide-based vaccines, including DNA and mRNA vaccines with their intracellular Ag synthesis, have been shown to be potent activators of a cytotoxic T cell response which is an important prerequisite for successful immunotherapy against many viral diseases and tumors [67], though intracellular delivery of mRNA vaccines to the cytosol of APCs is still not sufficiently well understood and remains somewhat a challenge to clinical translation for cancer immunotherapy [68].

6. NPs for overcoming immunosuppression

With great advances in immunology and oncology, several mechanisms, involving multiple immune components, have been identified to contribute to tumor immune escape, as summarized by Chabanon and coauthors as these including [69]: (1) reduction of MHC-I molecule expression in malignant cells, resulting in decreased antigen presentation and consequently reduced detection by CTLs; (2) induction of immune cell apoptosis by cancer cells through the expression of death signals; (3) release of a variety of immune-modulatory molecules such as IL6 and IL10 by tumor cells in the microenvironment to induce immunosuppressive Tregs while inhibiting the activity of CTLs; (4) secretion of TGF-β, COX-2 (cyclooxygenase-2), and PGE2 (prostaglandin E2) by tumor cells inhibiting DC differentiation and maturation while favoring the establishment of an immunosuppressive tumor microenvironment; (5) upregulated expression of immune checkpoint ligands to activate immune checkpoint receptors providing co-inhibitory signals to CD4+ and CD8+ T cells preventing them from building a specific antitumor immune response.

Among these elements involved in cancer resistance, immune checkpoints are regulators of the immune system to provide pathways crucial for self-tolerance and thus play an important

role both in the prevention of autoimmunity refraining the immune system from attacking cells indiscriminately under normal physiological conditions and in the regulation of immune reaction to avoid tissue damages during the pathogenic infection. Under normal conditions, immune checkpoints function via the interaction between a receptor expressed on T cells and its ligand located at the surface of APCs to generate a co-stimulatory signal, which triggers either the activation or inhibition of T cells. Presently, two major checkpoints have been clearly identified to regulate T cell activation: (i) the CD28/CTLA-4 axis, which activates T cells upon engagement of CD28 with CD80 and CD86, and conversely inhibits T cells when CTLA-4 is engaged and (ii) the PD-1 axis, which provides a strong inhibitory signal following binding of PD-L1 or PD-L2 to the PD-1 receptor [70]. Contrary to CTLA-4, PD-1 is thought to act predominantly in the tumor microenvironment, where PD-L1 is overexpressed by multiple cell types, including dendritic cells, M2 macrophages, and tumor-associated fibroblasts [71]. Thus, immune checkpoints and pathways, unfortunately, are also utilized by cancer cells as a key mechanisms to realize immune escape through upregulated expression of immune checkpoint ligands and as such deregulation of immune checkpoint signaling to suppress T cell activity in tumor microenvironment, a phenomenon that has been observed in multiple malignancies. Moreover, immune checkpoint molecules have been shown to promote the epithelial-mesenchymal transition of tumor cells and the acquisition of tumor-initiating potential and resistance to apoptosis and antitumor drugs, as well as the propensity to disseminate and metastasize, and thus have been increasingly considered as a crucial target for cancer immunotherapy given their potential for use in multiple types of cancers. Notably, as opposed to other immune-based approaches developed to fight cancers, immune checkpoint blockers (ICBs) have displayed significant therapeutic successes in many solid tumors and hematologic malignancies, as exampled by several anti-PD-(L)1-based drugs, such as the anti-CTLA-4 ipilimumab (by Bristol-Myers Squibb), the anti-PD-1 pembrolizumab (by Merck), and the anti-PD-L1 atezolizumab (by Genentech/Roche), durvalumab (by AstraZeneca/MedImmune), and avelumab (by Pfizer), all of which have already been approved for cancer immunotherapy [69].

However, with the current antibody-based immune checkpoint therapy, the nonspecific accumulation of antibody in the normal organs and tissues may ignite overreactive immune responses, which may even damage the body and cause severe side effects [72]; suggesting targeting delivery may provide beneficial effects even in the antibody-based immunotherapy. Recent studies have shown that a diverse set of NPs that have been engineered to improve delivery efficiency of immune checkpoint modulators which possess the potency in enhancement of the anticancer efficacy of the immune checkpoint blockade-based immunotherapy. Using a common procedure of water-in-oil-in-water emulsion, Wang's group formulated cationic NPs loaded with CTLA4 siRNA (siCTLA4) which was to modulate immune suppression mechanism [73]. The siCTLA4-NPs delivered siRNA into the T cells reducing mRNA and protein levels of CTLA4 upon the T cell activation in vitro and, when systemically given to mice, significantly increased the number of both CD4+ T cells and CD8+ T cells, whereas the number of CD4+ FOXP3+ regulatory T cells were decreased, resulting in the inhibited tumor growth and prolonged survival rate of B16 mouse melanoma model. PD-L1 is expressed on a variety of tumor cells, such as melanoma, NSCLC, ovarian cancer, head and neck cancer, B cell lymphoma, and thymic cancer and therefore is another attractive target for immune checkpoint modulation, which can be realized using tumor-targeted delivery system loaded

with relevant functional molecules. Yang et al. engineered folic acid-modified NPs with polyethyleneimine (PEI) derivatives and demonstrated that PD-L1 siRNA-loaded PEI NPs efficiently inhibited PD-L1 expression on SKOV-3-Luc tumor cells, resulting in sensitizing tumor cells to T cell killing in vitro [74]. Considering cancer recurrence after surgical resection remains still a significant challenge and platelets can accumulate in wound sites and interact with circulating tumor cells (CTCs) triggering inflammation and repair processes in the remaining tumor microenvironment, Gu's group engineered the anti-PD-L1 antibody-conjugated platelets (P-aPDL1) which were employed to reduce postsurgical tumor recurrence and metastasis [75]. In mouse models bearing partially removed primary melanomas (B16-F10) or 4T1 (triple-negative breast carcinomas), B16-F10 effectively released anti-PD-L1 upon platelet activation by platelet-derived microparticles and remarkably prolonged overall mouse survival after surgery by reducing the risk of cancer regrowth and metastatic spread, suggesting engineered platelets an efficient VADS which can facilitate the delivery of the immunotherapeutic anti-PD-L1 to the surgical bed and target CTCs in the bloodstream to improve the objective response rate.

Summarily, the VADSs based on various NPs that are engineered to bear therapeutic functions are promising in targeted delivery of the immunomodulatory agents to offset the immunosuppressive effects generated in tumor microenvironment and to rehabilitate the defensive immunity, maximizing the efficacy of cancer immunotherapy while minimizing side effects.

7. Conclusions

In recent years various types of NPs have been designed as a VADS for delivery of vaccines that are aimed for cancer immunotherapies and have shown great promise in curing refractory tumors which can never be obtained by conventional clinical measures, such as chemotherapeutics, surgery, and radiation. The NP-based cancer VADS possesses numerous advantages, including high safety profile and thus good compliance, high stability, diverse administration routes, and ease in modification with functional molecules as well as large-scale production, and bears also disadvantages including mainly relatively weak immunostimulatory capacity and low intracellular especially intranuclear delivery efficiency, which may be hopefully overcome by elaborate design with adjuvants such as PRRas and multifunctional molecules. Nevertheless, the NP-based cancer VADS proves able to successfully elicit antitumor immunity both in vitro and in vivo through, in particular, targeting APCs and draining lymph nodes, engendering lysosome escape, and modulating immunosuppression and represents new directions in developing efficient tools for cancer immunotherapy.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (Grant numbers 81703449) and partially by the Department of Science & Technology of Anhui Province for the Natural Science Research Project (Grant number 1708085QH195).

Conflict of interest

All the authors declared no conflict of interests.

Author details

Aizong Shen[1]*, Rui Qian[2], Ting Liu[2], Qingchuan Liu[3], Bin Liu[1] and Zhangbao Wu[1]

*Address all correspondence to: aizongshen@163.com

1 Department of Pharmacy, Anhui Provincial Hospital, Hefei, Anhui Province, China

2 School of Pharmacy, Anhui Medical University, Hefei, China

3 School of Biomedical Engineering, Hefei University of Technology, Hefei, China

References

[1] Yang Y. Cancer immunotherapy: Harnessing the immune system to battle cancer. The Journal of Clinical Investigation. 2015;**125**(9):3335-3337

[2] Melero I, Gaudernack G, Gerritsen W, Huber C, Parmiani G, Scholl S, et al. Therapeutic vaccines for cancer: An overview of clinical trials. Nature Reviews. Clinical Oncology. 2014;**11**(9):509-524

[3] Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. Nature. 2011; **480**(7378):480-489

[4] Fesnak AD, June CH, Levine BL. Engineered T cells: The promise and challenges of cancer immunotherapy. Nature Reviews Cancer. 2016;**16**(9):566-581

[5] Chen DS, Mellman I. Oncology meets immunology: The cancer-immunity cycle. Immunity. 2013;**39**(1):1-10

[6] Fan Y, Moon JJ. Nanoparticle drug delivery systems designed to improve cancer vaccines and immunotherapy. Vaccine. 2015;**3**(3):662-685

[7] Cubillos-Ruiz JR, Silberman PC, Rutkowski MR, Chopra S, Perales-Puchalt A, Song M, et al. ER stress sensor XBP1 controls anti-tumor immunity by disrupting dendritic cell homeostasis. Cell. 2015;**161**(7):1527-1538

[8] Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: Moving beyond current vaccines. Nature Medicine. 2004;**10**(9):909-915

[9] Yoon HY, Selvan ST, Yang Y, Kim MJ, Yi DK, Kwon IC, et al. Engineering nanoparticle strategies for effective cancer immunotherapy. Biomaterials. 2018;**178**:597-607

[10] Wang T, Zhen Y, Ma X, Wei B, Wang N. Phospholipid bilayer-coated aluminum nanoparticles as an effective vaccine adjuvant-delivery system. ACS Applied Materials & Interfaces. 2015;**7**(12):6391-6396

[11] Wang N, Wang T. Preparation of multifunctional liposomes as a stable vaccine delivery-adjuvant system by procedure of emulsification-lyophilization. Methods in Molecular Biology. 2016;**1404**:635-649

[12] Wang N, Zhen Y, Jin Y, Wang X, Li N, Jiang S, et al. Combining different types of multifunctional liposomes loaded with ammonium bicarbonate to fabricate microneedle arrays as a vaginal mucosal vaccine adjuvant-dual delivery system (VADDS). Journal of Controlled Release. 2017;**246**:12-29

[13] Wang N, Wang T, Zhang M, Chen R, Niu R, Deng Y. Mannose derivative and lipid A dually decorated cationic liposomes as an effective cold chain free oral mucosal vaccine adjuvant-delivery system. European Journal of Pharmaceutics and Biopharmaceutics. 2014;**88**(1):194-206

[14] Wang T, Zhen Y, Ma X, Wei B, Li S, Wang N. Mannosylated and lipid A-incorporating cationic liposomes constituting microneedle arrays as an effective oral mucosal HBV vaccine applicable in the controlled temperature chain. Colloids and Surfaces B: Biointerfaces. 2015;**126**:520-530

[15] Sunshine JC, Green JJ. Nanoengineering approaches to the design of artificial antigen-presenting cells. Nanomedicine. 2013;**8**(7):1173-1189

[16] Fan Y, Kuai R, Xu Y, Ochyl LJ, Irvine DJ, Moon JJ. Immunogenic cell death amplified by co-localized adjuvant delivery for cancer immunotherapy. Nano Letters. 2017; **17**(12):7387-7393

[17] Stephan MT, Irvine DJ. Enhancing cell therapies from the outside in: Cell surface engineering using synthetic nanomaterials. Nano Today. 2011;**6**(3):309-325

[18] Jung S, Unutmaz D, Wong P, Sano G, De los Santos K, Sparwasser T, et al. In vivo depletion of CD11c+ dendritic cells abrogates priming of CD8+ T cells by exogenous cell-associated antigens. Immunity. 2002;**17**(2):211-220

[19] Villadangos JA, Schnorrer P. Intrinsic and cooperative antigen-presenting functions of dendritic-cell subsets in vivo. Nature Reviews Immunology. 2007;**7**(7):543-555

[20] Cresswell P, Ackerman AL, Giodini A, Peaper DR, Wearsch PA. Mechanisms of MHC class I-restricted antigen processing and cross-presentation. Immunological Reviews. 2005;**207**:145-157

[21] Munz C. Live long and prosper for antigen cross-presentation. Immunity. 2015; **43**(6):1028-1030

[22] Zehner M, Marschall AL, Bos E, Schloetel JG, Kreer C, Fehrenschild D, et al. The translocon protein Sec61 mediates antigen transport from endosomes in the cytosol for cross-presentation to CD8(+) T cells. Immunity. 2015;**42**(5):850-863

[23] Mantegazza AR, Savina A, Vermeulen M, Perez L, Geffner J, Hermine O, et al. NADPH oxidase controls phagosomal pH and antigen cross-presentation in human dendritic cells. Blood. 2008;**112**(12):4712-4722

[24] Joffre OP, Segura E, Savina A, Amigorena S. Cross-presentation by dendritic cells. Nature Reviews Immunology. 2012;**12**(8):557-569

[25] Jongbloed SL, Kassianos AJ, McDonald KJ, Clark GJ, Ju X, Angel CE, et al. Human CD141+ (BDCA-3)+ dendritic cells (DCs) represent a unique myeloid DC subset that cross-presents necrotic cell antigens. The Journal of Experimental Medicine. 2010;**207**(6):1247-1260

[26] Trapani JA, Smyth MJ. Functional significance of the perforin/granzyme cell death pathway. Nature Reviews Immunology. 2002;**2**(10):735-747

[27] Zhu J, Yamane H, Paul WE. Differentiation of effector CD4 T cell populations. Annual Review of Immunology. 2010;**28**:445-489

[28] Bailey SR, Nelson MH, Himes RA, Li Z, Mehrotra S, Paulos CM. Th17 cells in cancer: The ultimate identity crisis. Frontiers in Immunology. 2014;**5**:276

[29] Schoenberger SP, Toes RE, van der Voort EI, Offringa R, Melief CJ. T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. Nature. 1998;**393**(6684):480-483

[30] Huster KM, Busch V, Schiemann M, Linkemann K, Kerksiek KM, Wagner H, et al. Selective expression of IL-7 receptor on memory T cells identifies early CD40L-dependent generation of distinct CD8+ memory T cell subsets. Proceedings of the National Academy of Sciences of the United States of America. 2004;**101**(15):5610-5615

[31] Qi Y, Li RM, Kong FM, Li H, Yu JP, Ren XB. How do tumor stem cells actively escape from host immunosurveillance? Biochemical and Biophysical Research Communications. 2012;**420**(4):699-703

[32] Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: From immunosurveillance to tumor escape. Nature Immunology. 2002;**3**(11):991-998

[33] Lindau D, Gielen P, Kroesen M, Wesseling P, Adema GJ. The immunosuppressive tumour network: Myeloid-derived suppressor cells, regulatory T cells and natural killer T cells. Immunology. 2013;**138**(2):105-115

[34] Butt AQ, Mills KH. Immunosuppressive networks and checkpoints controlling antitumor immunity and their blockade in the development of cancer immunotherapeutics and vaccines. Oncogene. 2014;**33**(38):4623-4631

[35] Saha A, Kaul R, Murakami M, Robertson ES. Tumor viruses and cancer biology: Modulating signaling pathways for therapeutic intervention. Cancer Biology & Therapy. 2010;**10**(10):961-978

[36] Pinschewer DD. Virally vectored vaccine delivery: Medical needs, mechanisms, advantages and challenges. Swiss Medical Weekly. 2017;**147**:w14465

[37] Pol J, Bloy N, Buque A, Eggermont A, Cremer I, Sautes-Fridman C, et al. Trial watch: Peptide-based anticancer vaccines. Oncoimmunology. 2015;**4**(4):e974411

[38] Hailemichael Y, Overwijk WW. Peptide-based anticancer vaccines: The making and unmaking of a T-cell graveyard. Oncoimmunology. 2013;**2**(7):e24743

[39] Schwartzentruber DJ, Lawson DH, Richards JM, Conry RM, Miller DM, Treisman J, et al. gp100 peptide vaccine and interleukin-2 in patients with advanced melanoma. The New England Journal of Medicine. 2011;**364**(22):2119-2127

[40] Dorta-Estremera S, Chin RL, Sierra G, Nicholas C, Yanamandra AV, Mahalakshmi Krishna NS, et al. Mucosal HPV E6/E7 peptide vaccination in combination with immune checkpoint modulation induces regression of HPV+ oral cancers. Cancer Research. 2018. DOI: 10.1158/0008-5472.CAN-18-0892. [Epub ahead of print]

[41] Obara W, Hara I, Kato Y, Kato R, Inoue K, Sato F, et al. Immunotherapy with cancer peptides in combination with intravesical bacillus Calmette-Guerin for patients with non-muscle invasive bladder cancer. Cancer Immunology, Immunotherapy. 2018. DOI: 10.1007/s00262-018-2197-x. [Epub ahead of print]

[42] Sorkin A, Von Zastrow M. Signal transduction and endocytosis: Close encounters of many kinds. Nature Reviews. Molecular Cell Biology. 2002;**3**(8):600-614

[43] Yuba E, Tajima N, Yoshizaki Y, Harada A, Hayashi H, Kono K. Dextran derivative-based pH-sensitive liposomes for cancer immunotherapy. Biomaterials. 2014;**35**(9):3091-3101

[44] Scott EA, Stano A, Gillard M, Maio-Liu AC, Swartz MA, Hubbell JA. Dendritic cell activation and T cell priming with adjuvant- and antigen-loaded oxidation-sensitive polymersomes. Biomaterials. 2012;**33**(26):6211-6219

[45] Nakamura T, Ono K, Suzuki Y, Moriguchi R, Kogure K, Harashima H. Octaarginine-modified liposomes enhance cross-presentation by promoting the C-terminal trimming of antigen peptide. Molecular Pharmaceutics. 2014;**11**(8):2787-2795

[46] Ahn S, Lee IH, Kang S, Kim D, Choi M, Saw PE, et al. Gold nanoparticles displaying tumor-associated self-antigens as a potential vaccine for cancer immunotherapy. Advanced Healthcare Materials. 2014;**3**(8):1194-1199

[47] Kakwere H, Ingham ES, Allen R, Mahakian LM, Tam SM, Zhang H, et al. Toward personalized peptide-based cancer nanovaccines: A facile and versatile synthetic approach. Bioconjugate Chemistry. 2017;**28**(11):2756-2771

[48] Kuai R, Ochyl LJ, Bahjat KS, Schwendeman A, Moon JJ. Designer vaccine nanodiscs for personalized cancer immunotherapy. Nature Materials. 2017;**16**(4):489-496

[49] Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: A leading role for STAT3. Nature Reviews Cancer. 2009;**9**(11):798-809

[50] Zeng B, Li H, Liu Y, Zhang Z, Zhang Y, Yang R. Tumor-induced suppressor of cytokine signaling 3 inhibits toll-like receptor 3 signaling in dendritic cells via binding to tyrosine kinase 2. Cancer Research. 2008;**68**(13):5397-5404

[51] Luo Z, Wang C, Yi H, Li P, Pan H, Liu L, et al. Nanovaccine loaded with poly I:C and STAT3 siRNA robustly elicits anti-tumor immune responses through modulating tumor-associated dendritic cells in vivo. Biomaterials. 2015;**38**:50-60

[52] Kumar H, Kawai T, Akira S. Pathogen recognition by the innate immune system. International Reviews of Immunology. 2011;**30**(1):16-34

[53] Zhang W, An M, Xi J, Liu H. Targeting CpG adjuvant to lymph node via dextran conjugate enhances antitumor immunotherapy. Bioconjugate Chemistry. 2017;**28**(7):1993-2000

[54] Xu Z, Ramishetti S, Tseng YC, Guo S, Wang Y, Huang L. Multifunctional nanoparticles co-delivering Trp2 peptide and CpG adjuvant induce potent cytotoxic T-lymphocyte response against melanoma and its lung metastasis. Journal of Controlled Release. 2013;**172**(1):259-265

[55] Zhu G, Lynn GM, Jacobson O, Chen K, Liu Y, Zhang H, et al. Albumin/vaccine nanocomplexes that assemble in vivo for combination cancer immunotherapy. Nature Communications. 2017;**8**(1):1954

[56] Hailemichael Y, Dai Z, Jaffarzad N, Ye Y, Medina MA, Huang XF, et al. Persistent antigen at vaccination sites induces tumor-specific CD8(+) T cell sequestration, dysfunction and deletion. Nature Medicine. 2013;**19**(4):465-472

[57] Kim SY, Noh YW, Kang TH, Kim JE, Kim S, Um SH, et al. Synthetic vaccine nanoparticles target to lymph node triggering enhanced innate and adaptive antitumor immunity. Biomaterials. 2017;**130**:56-66

[58] Molino NM, Neek M, Tucker JA, Nelson EL, Wang SW. Viral-mimicking protein nanoparticle vaccine for eliciting anti-tumor responses. Biomaterials. 2016;**86**:83-91

[59] Bachmann MF, Jennings GT. Vaccine delivery: A matter of size, geometry, kinetics and molecular patterns. Nature Reviews Immunology. 2010;**10**(11):787-796

[60] Benne N, van Duijn J, Kuiper J, Jiskoot W, Slutter B. Orchestrating immune responses: How size, shape and rigidity affect the immunogenicity of particulate vaccines. Journal of Controlled Release. 2016;**234**:124-134

[61] Pol J, Bloy N, Obrist F, Eggermont A, Galon J, Herve Fridman W, et al. Trial watch: DNA vaccines for cancer therapy. Oncoimmunology. 2014;**3**(1):e28185

[62] Rice J, Ottensmeier CH, Stevenson FK. DNA vaccines: Precision tools for activating effective immunity against cancer. Nature Reviews Cancer. 2008;**8**(2):108-120

[63] Shu D, Shu Y, Haque F, Abdelmawla S, Guo P. Thermodynamically stable RNA three-way junction for constructing multifunctional nanoparticles for delivery of therapeutics. Nature Nanotechnology. 2011;**6**(10):658-667

[64] Cole G, Ali AA, McCrudden CM, McBride JW, McCaffrey J, Robson T, et al. DNA vaccination for cervical cancer: Strategic optimisation of RALA mediated gene delivery from a biodegradable microneedle system. European Journal of Pharmaceutics and Biopharmaceutics. 2018;**127**:288-297

[65] Nanda RK, Hajam IA, Edao BM, Ramya K, Rajangam M, Chandra Sekar S, et al. Immunological evaluation of mannosylated chitosan nanoparticles based foot and mouth

disease virus DNA vaccine, pVAC FMDV VP1-OmpA in guinea pigs. Biologicals. 2014; **42**(3):153-159

[66] Oberli MA, Reichmuth AM, Dorkin JR, Mitchell MJ, Fenton OS, Jaklenec A, et al. Lipid nanoparticle assisted mRNA delivery for potent cancer immunotherapy. Nano Letters. 2017;**17**(3):1326-1335

[67] Melief CJ, van Hall T, Arens R, Ossendorp F, van der Burg SH. Therapeutic cancer vaccines. The Journal of Clinical Investigation. 2015;**125**(9):3401-3412

[68] Kauffman KJ, Webber MJ, Anderson DG. Materials for non-viral intracellular delivery of messenger RNA therapeutics. Journal of Controlled Release. 2016;**240**:227-234

[69] Chabanon RM, Pedrero M, Lefebvre C, Marabelle A, Soria JC, Postel-Vinay S. Mutational landscape and sensitivity to immune checkpoint blockers. Clinical Cancer Research. 2016;**22**(17):4309-4321

[70] Topalian SL, Drake CG, Pardoll DM. Immune checkpoint blockade: A common denominator approach to cancer therapy. Cancer Cell. 2015;**27**(4):450-461

[71] Chen DS, Irving BA, Hodi FS. Molecular pathways: Next-generation immunotherapy: Inhibiting programmed death-ligand 1 and programmed death-1. Clinical Cancer Research. 2012;**18**(24):6580-6587

[72] Postow MA, Sidlow R, Hellmann MD. Immune-related adverse events associated with immune checkpoint blockade. The New England Journal of Medicine. 2018;**378**(2):158-168

[73] Li SY, Liu Y, Xu CF, Shen S, Sun R, Du XJ, et al. Restoring anti-tumor functions of T cells via nanoparticle-mediated immune checkpoint modulation. Journal of Controlled Release. 2016;**231**:17-28

[74] Teo PY, Yang C, Whilding LM, Parente-Pereira AC, Maher J, George AJ, et al. Ovarian cancer immunotherapy using PD-L1 siRNA targeted delivery from folic acid-functionalized polyethylenimine: Strategies to enhance T cell killing. Advanced Healthcare Materials. 2015;**4**(8):1180-1189

[75] Chao Wang WS, Ye Y, Hu Q, Bomba HN, Gu Z. In situ activation of platelets with checkpoint inhibitors for post-surgical cancer immunotherapy. Nature Biomedical Engineering. 2017;**1**(1):1-10

IntechOpen