Review

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Recent development and applications of nanomaterials for cancer immunotherapy

Abstract: Immunotherapy, which utilizes the patient's own immune system to fight against cancer, further results in durable antitumor responses and reduces metastasis and recurrence, has become one of the most effective and important cancer therapies along with surgery, radiotherapy, and chemotherapy. Nanomaterials with the advantages of large specific surface, delivery function, and controllable surface chemistry are used to deliver antigens or adjuvants, or both, help to boost immune responses with the imaging function or just act as adjuvants themselves and modulate tumor microenvironment (TME). In this review, recent development and applications of nanomaterials for cancer immunotherapy including delivery systems based on nanomaterials, uniting imaging, self-adjuvants, targeting functions, artificial antigen presenting cells, and TME modulation are focused and discussed.

Keywords: nanomaterials, cancer immunotherapy, cancer nano-vaccine, tumor microenvironment

1 Introduction

Cancer immunotherapy has drawn a great attention due to the unique characteristics of the immune system targeting tumor cells without sacrificing healthy cells, and the feature of long-term control of cancer in the response population, leading to a suppression of cancer metastasis and recurrence, which is never seen in other therapies like surgery, radiotherapy, or chemotherapy [1,2]. Bacterial toxins, which were used for cancer treatment by William Coley in 1890, was a rudiment of

cancer immunotherapy [3], then the Sipuleucel-T (APC 8015), which was the first dendritic vaccine-based cellular immunotherapy, and ipilimumab, which was the first checkpoint inhibitor and a fully monoclonal antibody, were approved by the US Food and Drug Administration (FDA) in 2010 and 2011, respectively [4,5]. Since then, the development of cancer immunotherapy has been dramatically induced and developed, but there are still many challenges of enhancing the therapeutic benefits and reducing side effects.

Nanomaterials with a size at least one dimension between 1 and 200 nm are being practically useful for cancer diagnosis, prevention, and treatment in novel radiotherapy, chemotherapy, photodynamic therapy, gene therapy, and immunotherapy [6–8]. For immunotherapy, multiple nanomaterial-based drug delivery systems including liposomes, polymeric micelles, gold nanoparticles, and carbon nanotube are being used to deliver antigens or adjuvants or both simultaneously [9–12]. Free antigens with relatively low efficiency in cross-presentation are normally poorly presented to major histocompatibility complex (MHC) class I molecules due to the micropinocytosis internalization mechanism, and they could be rapidly eliminated and degraded after being administered subcutaneously or intradermally [13–15]. However, the nanoparticles that are administered intradermally, intramuscularly, and intraperitoneally could enhance the antigen cross-presentation, resulting in significantly higher humoral and cellular immune responses [16–18]. Furthermore, nanomaterials with targeting ligands being preloaded on the surface are easy to be taken up by the targeting cells [19,20]. Additionally, nanomaterials could deliver both antigens and adjuvants at the same time to avoid the immune tolerance caused by the lack of "danger signals," which are generated by specific adjuvants to dendrite cells (DCs) encountering antigens when delivering antigens and adjuvants separately [21,22]. The most commonly used nanomaterial-based delivery systems include liposome-based system, polymeric nanomaterial-based system, gold nanoparticle-based system, carbon-based system, and porous silicon-based system [23–25].

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It would be an advantage if the nanomaterial-based delivery systems are equipped with additional imaging function to monitor the treatment effect or act as adjuvants themselves without co-delivering antigens and adjuvants to decrease the difficulty of delivery while increasing the immune response. It has been demonstrated that gold nanoparticles, Fe_3O_4-based nanoparticles, and upconversion nanoparticles are imaging functionalized for CT and photoacoustic imaging, magnetic resonance imaging (MRI), and upconversion luminescence, respectively [26,27]. Poly (D,L-lactide-co-glycolide) (PLGA), carbon nanotubes, acetylated dextran, and graphene-based nanomaterials have been proved to be the self-adjuvants functionalized to induce the uptake of antigens by antigen-presenting cells (APCs) and increase the activation of APCs, which further develops more effective antitumor immune response [28,29]. Nanomaterials can also play a more important role in regulating the immunosuppressive tumor microenvironment (TME) themselves.

In this review, we will focus on the recent development and applications of nanomaterials for cancer immunotherapy. Some perspectives of applying nanomaterials to immunotherapy will be offered through summarizing and discussing the recent development.

2 Rationale of the functions of nanomaterials for immunotherapy

Cancer immunotherapy is designed to generate a self-sustaining cycle of cancer immunity, which is called "cancer immunity cycle" (Figure 1) [30,31], enabling it to amplify and propagate without generating unrestrained autoimmune inflammatory responses [31]. There are seven steps being classified as two parts of systematic-based and local-based immunotherapies for the cancer immunity cycle: (1) neoantigens are created, released, and captured by DCs; (2) the captured antigens on MHCI and MHCII molecules are processed and presented to T cells; (3) the DCs prime and activate immature T cells in the draining lymph nodes; (4) the activated effector T cells traffic to the tumor site; (5) the activated effector T cells infiltrate into tumors; (6) the T cells recognize cancer cells through T-cell receptor; and (7) the effector T cells kill the target cancer cells by inducing the apoptotic pathways. The killing of cancer cells releases additional tumor-associated antigens (step 1) to repeat and enhance the immunity cycle afterward [13,30].
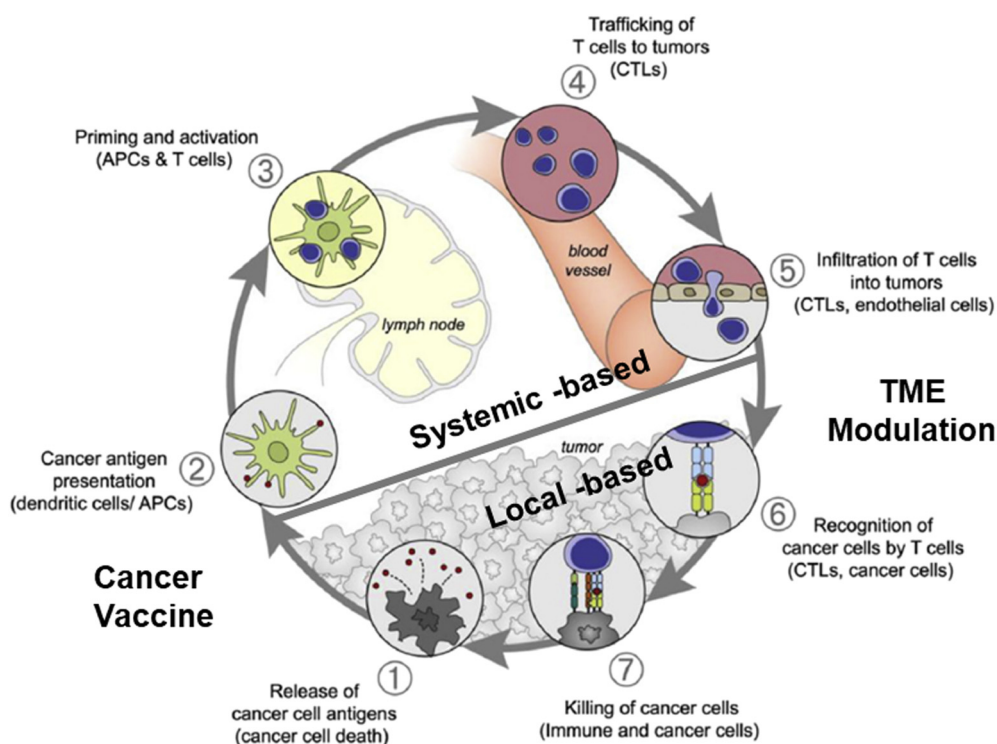

Figure 1: The cancer immunity cycle.

During the immunotherapy treatment, the cancer immunity cycle might be blocked at one or more steps, leading to insufficient therapeutic effect or even immune escape. In addition, the immunosuppressive TME could be generated to impede anti-tumor immune responses. The cancer-associated fibroblasts, tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), regulatory T cells (Tregs), and some cytokines, which constitute the immunosuppressive TME, could facilitate the growth of tumor cells and protect them from being eliminated [13,32–34].

In consideration of the limitations of cancer immunotherapy or cancer immunity cycle, nanomaterials are used to overcome the limitations and develop immunotherapies more effectively in terms nano-vaccine and TME modulation. The functions of nanomaterials are listed in Table 1. For the period of cancer vaccine functionalizing, nanomaterials could be used to deliver antigen or adjuvants, or both, act as self-adjuvants, conduct lymph node drainage, target DCs, and present antigens. For TME modulation period, nanomaterials could be used to target immune checkpoints, soluble mediators, TAMs, MDSCs, Tregs, and tumor-associated fibroblasts (TAFs) [35–37]. With the specific properties of antigens and/or adjuvants protection and delivery, large specific surface area, controllable surface chemistry and size, multifunction, and self-adjuvants, nanomaterials are being widely used in cancer immunotherapy by promoting the cancer immunity cycle [38]. The rationale of each function is summarized as follows:

(1) Delivery of antigens: delivery of antigens or adjuvants is one of the most important functions applied at the first step of the immunity cycle. The tumor antigens are capable of generating the immune response, and adjuvants are capable of generating "danger signals" to assist to stimulate the maturation of DCs. In this way, antigens and adjuvants are the crucial parts to initiate or reinitiate the immunity cycle. But the antigens and adjuvants are easy to be degraded or failed to be taken up by DCs through the conventional delivery methods, which eventually leads to the failure of generating the immune response. To increase the delivery efficiency, various kinds of nanomaterials are used to encapsulate the antigens or adjuvants or both to protect them from degradation to generate the immune response.

(2) Co-encapsulation and co-delivery: delivering the antigens and adjuvants separately might cause the immune tolerance because of the absence of "danger signals" generated by adjuvants. The nanomaterials that could co-encapsulate and co-delivery both the antigens and the adjuvants at the same time could not only protect the antigens and adjuvants but also increase the efficiency of DCs uptake.

(3) Self-adjuvant: sometimes, the nanomaterials that are used for delivering the antigens could act as adjuvants themselves to generate the "danger signals" without co-delivering the specific adjuvants and then promote the antigen presentation, stimulating the immune response.

(4) Lymph node drainage: the nanomaterials encapsulated with antigens or adjuvants or both are needed to drain into the lymph node, where they could be presented by resident DCs. In this way, the size of the nanoparticle becomes the critical factor, which affects whether it could drain into lymph node and be taken up by DCs. The nanoparticles with a size of 40–50 nm are optimal for effective draining into lymph node to generate the immune response eventually.

(5) DCs uptake: similar to the lymph node drainage, the DCs uptake is also affected by the size of the nanoparticles, and it would decrease the uptake

Table 1: The function of nanomaterials for cancer immunotherapy

	Function period	
	Cancer vaccine	TME modulation
Functions of nanomaterials for cancer immunotherapy	Delivery of antigens and/or adjuvants	Immune checkpoint
	Self-adjuvants	Soluble mediators
	Lymph node drainage	Targeting TAMs
	DC uptake	Targeting MDSCs
	DC targeting	Targeting Tregs
	Antigen presentation artificial antigen presentation (aAPCs)	Targeting TAFs
	Peptide vaccine DNA and mRNA	

efficiency when the size of the nanoparticles exceeds 500 nm.

(6) DCs targeting: the nanoparticles could be modified with specific ligands for DCs surface receptors to increase the efficiency of DCs uptake.

(7) Antigen presentation: antigen presentation is a prerequisite to active the cytotoxic T lymphocyte (CTL). During the presentation process, soluble antigens are internalized by micropinocytosis, and they are cross-presented to MHCI with a low efficiency. In this way, the nanoparticles that encapsulate antigens could enter DCs through phagocytosis and present antigens with a higher efficiency.

(8) Peptide vaccine delivery: peptide vaccines are generally composed of two or more small fragments of antigen proteins and adjuvants. When the peptide vaccines are administered subcutaneously freely, the small fragments of antigens and adjuvants could be degraded and eliminated rapidly. In this way, nanomaterials are used to encapsulate and deliver the peptide vaccine to improve the efficiency.

(9) DNA and mRNA antigens delivery: DNA vaccines could mimic live infections compared with the conventional peptide vaccines. But the delivery efficiency is pretty low and the immunogenicity is weak when the DNA vaccines are administered as naked DNA, which could be degraded by nucleases. In this way, nanomaterials could be used to encapsulate and deliver the DNA-based vaccines.

(10) TME modulation: when immune response is successfully generated and T cells are effectively infiltrated into tumor cells, the T cells are ready to kill tumor cells. But the suppression of TME still could inhibit the immune cycle, leading to the failure of immunotherapy. Suppression of TME modulation is mainly caused by tumor cells, TAFs, TAMs, MDSCs, Tregs, etc. In this way, nanomaterials could be used to modulate the immunosuppressive TME by targeting TAFs, TAMs, MDSCs, and Tregs and inhibiting the soluble mediators.

(11) Targeting immune checkpoints: interactions between ligands and activated or inhibitory receptors could regulate T-cell activation or tolerance with multiple signals, including T cell receptor (TCR) recognition, CD28/B7 co-stimulation, and cytokine stimulation, and the co-stimulation signals are termed as immune checkpoints. Nanomaterials could be used to deliver the immune checkpoint modulators to tumors to complete the immunity cycle.

(12) Targeting soluble mediators: many cytokines and chemokines play an important role in immunosuppressive TME. Targeting the soluble mediators using nanomaterials encapsulated with small size molecule drugs or siRNA is an effective way to modulate TME.

(13) Targeting cellular mediators:

TAMs, which are immune cells, could be presented in a large amount in the TME. The immunoregulatory cytokines interleukin (IL)-12, IL-1b, IL-6, and tumor necrosis factor (TNF)-α, and TNF-β, which are produced by TAMs, could inhibit anti-cancer immune responses to promote the tumor growth. The nanomaterials that are surface-modified could be used to target and kill the TAMs.

Tregs are immunosuppressive T cells, which could control the severity of immune responses by inhibiting the activity of anti-tumor T-effector cells. Nanomaterials could be used to suppress or even kill the Tregs to generate tumor immunity.

MDSCs are tumor suppressor cells, which could control cancer inflammation by activating Tregs and suppressing other immune cells. Nanomaterials could deliver immunomodulators to eliminate MDSCs to improve cancer immunotherapy.

TAFs could mediate cancer sustaining and proliferative pathways and nanomaterials could easily deliver drugs into the tumor.

In this review, we will focus on the innovative researches and applications of nanomaterials in cancer immunotherapy. The most used nanomaterials and their pioneer works as well as some perspectives will be summarized and discussed.

3 Nanomaterials for cancer immunotherapy

Various kinds of nanomaterials including liposome-based, polymer-based, metallic-based and inorganic-based materials are being applied for cancer immunotherapy. The most used nanomaterials for cancer immunotherapy with various functions are listed in Table 2, with their advantages and disadvantages.

3.1 Poly(D,L-lactide-co-glycolide)

PLGA, which is approved by the FDA, is one of the most applied and important polymeric-based nanomaterials due to its multifunction, biodegradation and protection of agents for delivery or co-delivery of agents, self-adjuvant,

Table 2: Nanomaterials for cancer immunotherapy

Nanomaterials	Functions	Advantages	Disadvantages
PLGA [39–41]	Delivery and co-delivery system; peptide/DNA/mRNA and whole cell antigen delivery; target immune checkpoint, Tregs, TAFs, MDSCs; aAPCs	Multifunctional; versatile sizes, morphology and surface functionalization; high antigen loading; stronger cellular response and longer humoral response than liposome	Inflammation may be caused by degradation products; antigen degradation caused by encapsulation; difficult to produce monodisperse nanoparticle
Liposome [42–45]	Delivery and co-delivery system; target TAMs	Versatile chemistry; high biocompatibility; low immunogenicity; easy functionalization; antigen and adjuvant protection	Poor stability; poor immunogenic; poor drug loading efficiency
Gold nanoparticle [46–48]	Delivery system; imaging function; target Tregs	Versatile size; easy functionalization; biocompatible; additional imaging function	Nonbiodegradable; long-term toxicity
Fe_3O_4 nanoparticle [27,49,50]	Delivery system; imaging function; target immune checkpoint	Additional imaging function	Uptake efficiency strongly depends on the size of the nanoparticles
Carbon nanotube [51–53]	Delivery system; self-adjuvant; gene delivery; target Tregs	Additional self-adjuvant function; easy preparation and functionalization; biocompatible; bind macromolecules	Nonbiodegradable; low solubility; cytotoxicity; could lead to activation of the innate immune system and inflammation
Porous silicon [54–56]	Delivery system; self-adjuvant	Additional self-adjuvant function; easy preparation and functionalization; biocompatible; bind macromolecules	Nonbiodegradable; low solubility; cytotoxicity; could lead to activation of the innate immune system and inflammation
Iron dextran [57–59]	aAPCs	Biocompatible; enhance circulation time; innovative antigenic source	Stability
Quantum dot [57,60]	aAPCs	Biocompatible; enhance circulation time; innovative antigenic source	Stability

and applications for TME modulation. The behavior of PLGA nanoparticle could be changed dramatically when they are changed from micro-level to nano-level. The adsorption and adhesion of PLGA nanoparticles could be much stronger due to the great increase of the specific surface area. In this way, the drug absorption could be significantly improved. The preparation of PLGA nanoparticles includes emulsion or microemulsion polymerization, interfacial polymerization, and precipitation polymerization, which belong to "bottom-up" method starting with a monomer, emulsion solvent evaporation, emulsion diffusion, solvent displacement, slating-out, and spherical crystallization, which belong to "top-down" method starting with the preformed polymer [61].

3.1.1 Delivery and co-delivery of agent

Delivery or co-delivery of antigens or adjuvants or both is one of the most important functions of PLGA for cancer immunotherapy. The PLGA nanoparticles could encapsulate the agents that are necessary for initiating the immune cycle and deliver them for DCs uptake with the protection from being degraded. But the antigens might be degraded by co-encapsulation and inflammation might be caused by degradation products. The effects of antigen-loading methods on antigen exposure to the immune system and evaluating the resulting antigen-specific immune responses were investigated by Liu et al. through three classes of antigen adsorbed, antigen encapsulated, and antigen adsorbed/encapsulated PLGA hybrid nanoparticles as delivery systems, which were called "out," "in," and "both" nanoparticles, respectively [62]. The results indicated that the lysosomal escape and cross-presentation of antigens, which were exposed to "in" and "both" nanoparticles, were more efficient. Both adequate initial antigen exposure and long-term antigen persistence at the injection site for "both" nanoparticles were found to be more effective through *in vivo* experiments. Antigen-specific immune responses of "in" and "both" nanoparticles were higher than that of

"out" nanoparticles. The study also revealed that antigen entrapment was an important factor in modulating immune response of the antigens delivered by PLGA nanoparticles.

The efficacies of full-length ovalbumin (OVA) with adjuvant α-galactosylceramide (α-GalCer) and OVA with toll-like receptor (TLR) ligands in nanoparticles were investigated by Dölen et al. [63]. They found that the OVA + α-GalCer nanoparticles were more effective than the OVA + TLR nanoparticles in generating and stimulating antigen-specific cytotoxic T lymphocytes without the need for CD4+ T cell. Furthermore, the co-encapsulation of both α-GalCer and antigen in the PLGA nanoparticles was essential for T-cell responses. Moreover, the growth of established tumors could even be delayed by OVA + α-GalCer nanoparticles. In this way, the encapsulation of antigen and α-GalCer could be a choice for designing PLGA-based nanoparticles for cancer immunotherapy.

3.1.2 Peptide, DNA/mRNA, and whole cell antigen delivery

Peptide, which is composed of one or more small fragments of antigen proteins and adjuvants, DNA/mRNA vaccines, and whole cell antigens all are relatively large in size, easy to be degraded, and eliminated rapidly when being delivered nakedly.

They can be encapsulated and delivered by PLGA nanoparticles with protection to increase the delivery efficiency. Biodegradable PLGA nanoparticles were used to co-encapsulate anti-PD1 peptide (APP) and hollow gold nanoshell (HAuNs), which was termed as AA@PN, to construct a therapeutic strategy for immune check-point PD-1/PD-L1 blockade by Luo et al. [64]. They developed an administration strategy, which could maintain perdurable and controllable drug release by encapsulating the peptide for its sustained release, decreasing the frequency of administration and maintaining a constant drug concentration. The results showed that APP exhibited sustained release behavior from AA@PN within 40 days, which could be easily accelerated by near-infrared (NIR) laser (Figure 2).

Amir Kalvanagh et al. [65] encapsulated pcDNA encoding interferon (IFN)-λ1 (pIFN-λ1) with PLGA nanoparticles, which were spherical in shape with a mean diameter of 380 ± 3 nm to protect them against DNase enzyme action and increase the efficiency of gene delivery. The results showed that the encapsulation efficiency and loading capacity of PLGA nanoparticles were 75 ± 5% and 0.83 ± 0.06, respectively. The PLGA nanoparticles, which were bioactive, could be engulfed by RAW264.7 cells, and the pIFN-λ1 released from the nanoparticles sustainably. The study indicated that PLGA nanoparticles were an alternative choice for DNA delivery, which could be an alternative delivery system for cancer immunotherapy.
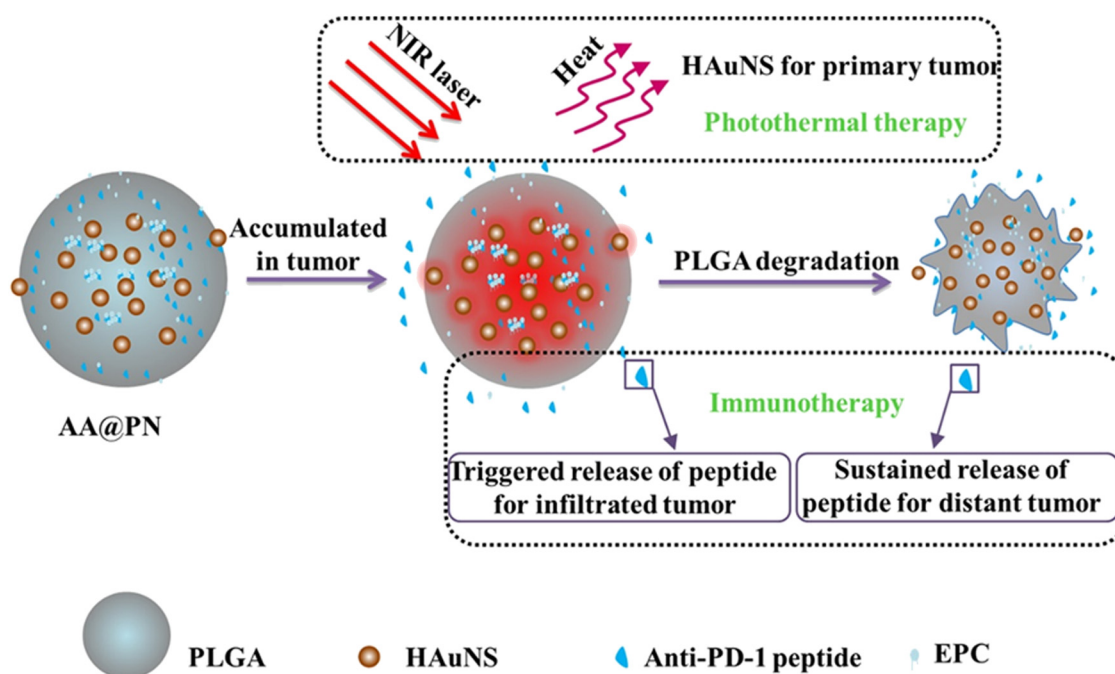

Figure 2: Illustration of preparation and structure of AA@PN and its combined therapeutic modalities.

3.1.3 Targeting immune checkpoint, Tregs, TAFs, and MDSCs

Targeting immune checkpoint, Tregs, TAFs, and MDSCs, which belong to TME modulation, are additional functions of PLGA nanomaterials for cancer immunotherapy. PLGA nanoparticles could target the molecules to modulate TME to help to complete the immune cycle. Indocyanine green, a photothermal agent, and imiquimod (R837), Toll-like-receptor-7 agonist, were coencapsulated with PLGA nanoparticles for checkpoint-blockade immunotherapy by Chen et al. [66]. They found that the tumor-associated antigens generated after photothermal tumor ablation showed vaccine-like functions, and in combination with checkpoint blockade using anti-cytotoxic T-lymphocyte antigen-4 (CTL4), the generated immunological responses could attract tumor cells (Figure 3). This PLGA-based nanoparticles with a strong immunological memory effect could be an alternative method to offer protection against tumor rechallenging after elimination of initial tumors.

Zhao et al. focused on the TME modulation and studied the delivery of methyl-2-cyano-3,12-dioxo-oleana-1,9(11)-dien-28-oate (CDDO-Me) by PLGA nanoparticles, and the results showed that the Tregs and MSDCs in a B16F10 melanoma model were dramatically decreased and TAFs were remodeled. The PLGA-CDDO-Me nanoparticles with lipid–calcium–phosphate (LCP) nanoparticles loaded with TRP2 peptide vaccine increased the antitumor efficacy than the bare TRP2 vaccine. The study further revealed that PLGA nanoparticles was an alternative drug delivery system for immune cells in the TME [67].

3.1.4 Artificial antigen-presenting cells

Biomimetic artificial antigen-presenting cells (aAPCs) could deliver the stimulatory signals including T-cell recognition signal and activation signal to activate and modulate the immune system. The PLGA-based nanoparticles could be synthesized for antigen presentation and T-cell activation *ex vivo* and *in vivo*. Meyer et al. [68] activated T cells *in vivo* using synthetic ellipsoidal PLGA aAPCs to demonstrate that nonspherical and anisotropic-shaped nanomaterials could take advantage of nonspecific cellular uptake. The results indicated that ellipsoidal PLGA-based aAPCs showed superior pharmacokinetic profiles over spherical aAPCs. Moreover, the live whole animal imaging analysis showed that ellipsoidal PLGA-based aAPCs remained in the periphery for longer periods of time than spherical aAPCs (Figure 4).

3.2 Liposomes

Liposomes are being widely used as delivery systems for specific drugs, genes, antibodies, antigens, adjuvants, and targeting functions due to their properties of biocompatibility with low toxicity, prevention for pharmaceuticals, smaller size, and easily changed charge and
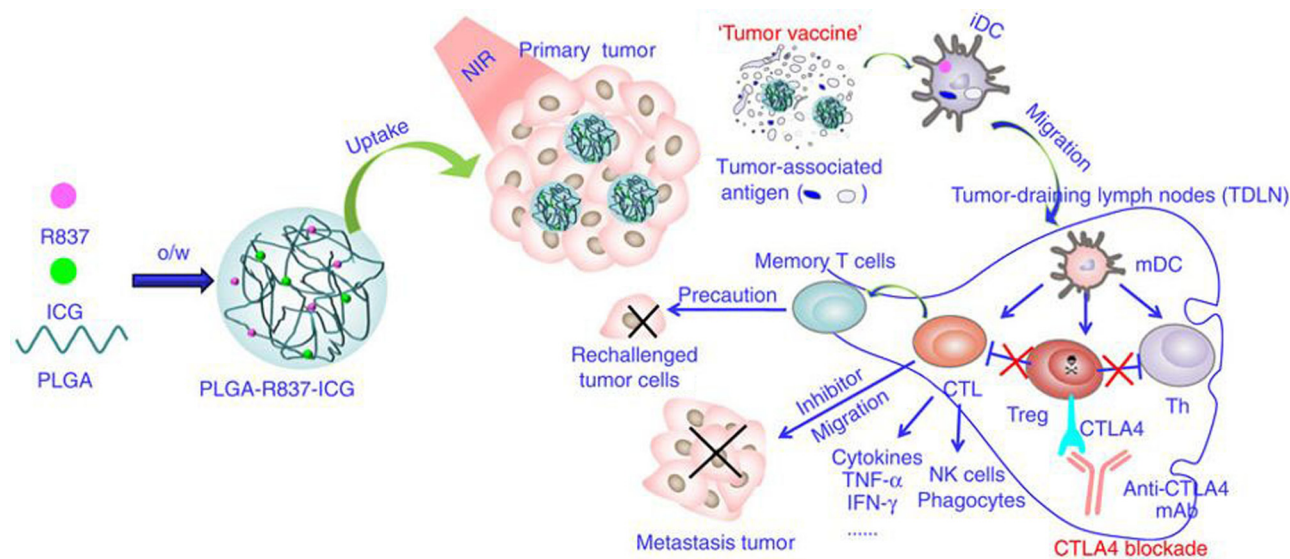
Figure 3: The mechanism of anti-tumor immune responses induced by the PLGA-based immunotherapy with checkpoint blockade.
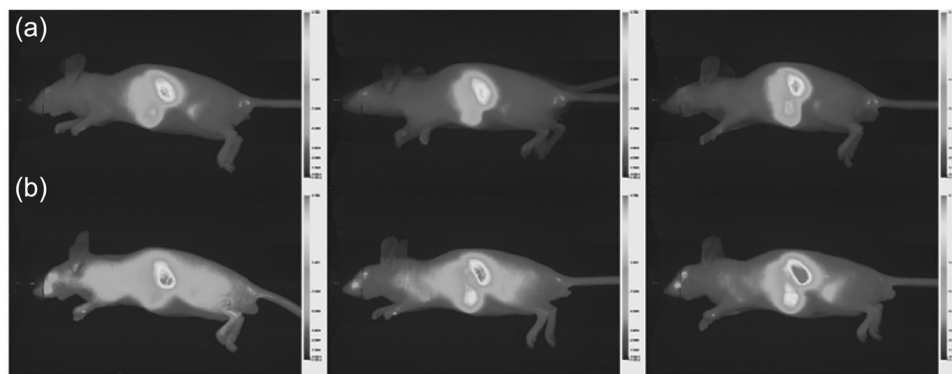
Figure 4: Nonspherical nano-ellipsoidal aAPCs have superior pharmacokinetics over nanospherical aAPCs: (a) spherical aAPCs and (b) ellipsoidal aAPCs.

surface chemistry. The size of the liposome nanoparticles mainly ranges from 25 nm to 2.5 μm. The liposome nanoparticles are composed of one or more bilayer membranes, which directly affect the amount of drug encapsulation along with the size of the nanoparticles. The liposome nanoparticles were prepared with mechanical dispersion method, solvent dispersion method, detergent removal method, etc., and the methods could be divided into four steps: drying down the lipids from organic solvent, then dispersing the lipid through aqueous media, purifying the resultant liposome, and last, analyzing the final product [69].

3.2.1 Delivery and co-delivery of agents

Liposomes are being widely used for cancer treatment due to their great biological compatibility with low cytotoxicity, offering protection for drugs from degradation or inactivation, easily changed size, charge, and chemistry of surface. The liposome nanoparticles could also deliver and co-deliver agents with the protection to increase the deliver efficiency. But the drug loading efficiency of the liposome nanoparticles is relatively low compared with that of the PLGA nanoparticles.

Immunoliposome co-delivery of bufalin and anti-CD40 was designed by Li et al. to induce therapeutic efficacy without systemic side effects [70]. The results demonstrated that bufalin liposomes with anti-CD40 antibody exhibited enhanced cytotoxicity compared with bufalin alone. The liposome delivery system exerted immune modulation through allowing anti-CD40 to be presented to APCs for antitumor effects. The prolonged release period at the tumor site could block the system toxicity.

As a new class of adjuvant for cancer treatment, cyclic di-GMP (c-di-GMP), a ligand of the stimulator of

interferon genes (STING) signal pathway, is widely employed for cancer immunotherapy. But the delivery of c-di-GMP remains a technology problem. Nakamura et al. loaded the c-di-GMP into YSK05 lipid-containing liposomes to deliver it to the cytosol effectively [71]. The YSK05-liposomes (c-di-GMP/YSK05-Lip) could induce the production of type I interferon with the activation of natural killer (NK) cells, which could lead to an antitumor effect in a lung metastasis. The results represented the use of a potential new adjuvant system in immunotherapy.

Cruz et al. encapsulated a fragment of the TAT NY-ESO-1 with a T-helper peptide into liposome nanoparticles as a nano-vaccine targeting the Fcγ-receptor to improve the immunological response against tumors [72]. The results showed that the liposome nanoparticles encapsulated with the peptide adjuvants could induce the most potent immunological response, and the liposome-based targeted vaccine could be an effective method to activate and deliver antigens to DCs to improve the immunotherapeutic response.

3.2.2 Targeting TAMs

In the TME modulation period, the liposome nanoparticles could mainly target TAMs, which show much less targets compared with the PLGA nanomaterials. The lipid-based nanoparticles, which were functionalized with fusion peptide coencapsulating M2pep and α-peptide, were used as the siRNA delivery system for specific elimination of M2-like TAMs by Qian et al. [73]. The lipid-based delivery system resulted in decreasing tumor size and prolonging survival, and at the same time, the nanoparticles increased the expression of immunostimulative cytokines IL-12 and IFN-γ and decreased T cell infiltration in the TME.

Zhou et al. used epirubicin-loaded liposomes encapsulated with synthesized sialic acid–cholesterol conjugate (SA-CH) for cancer immunotherapy of targeting TAMs, which was termed as EPI-SAL. The results indicated that EPI-SAL could improve the delivery of EPI to TAMs, reduce the number of TAMs in tumor-bearing mice, provide the strongest antitumor activity, and prolong the lifespan of tumor-bearing mice with decreased systemic toxicity. The research also indicated that EPI-loaded liposomes encapsulated with SA-CH could be an effective treatment for targeting TAMs for cancer immunotherapy. The delivery system targeting TAMs is shown in Figure 5 [74].
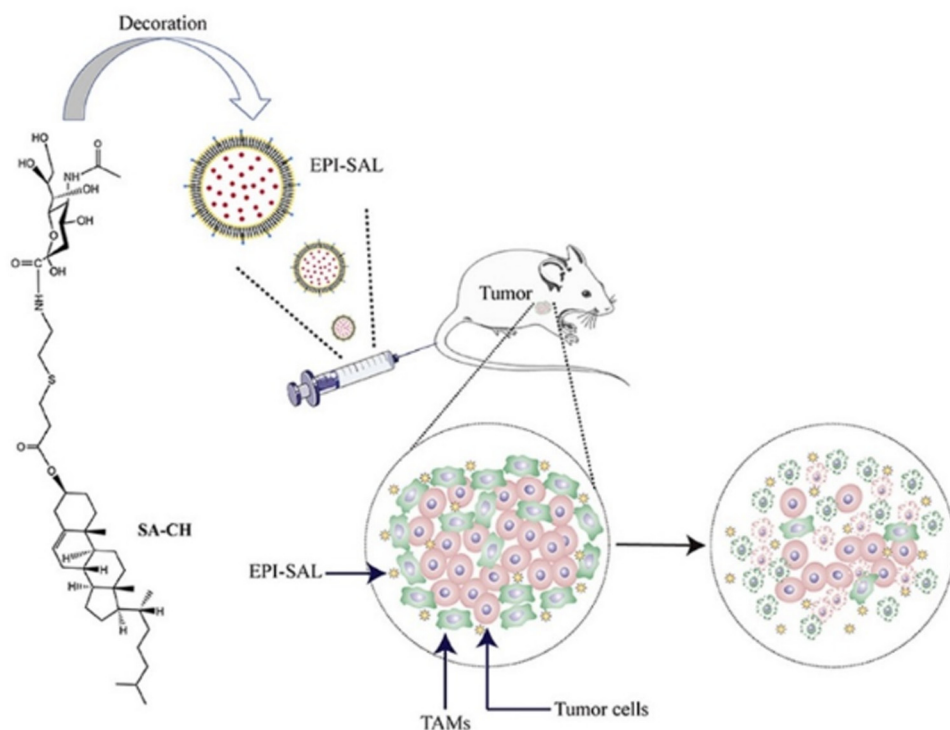
3.3 Gold nanoparticles

Gold nanoparticles have gained a lot of attention in cancer immunotherapy and imaging because they are biocompatible and bioinert, have versatile sizes and shapes, and are easily controlled by many synthetic methods. But the non-biodegradation and low-term toxicity become the limitations for their wide use. The size of the gold nanoparticles could be down to 1.5 nm and a wide array of solution are being used for preparation including $HAuCl_4$ with citric acid in boiling water, tetraoctylammonium bromide as the phase transfer reagent and sodium borohydride as the reducing agent, tetrachloroauric acid solution, and TiO_2 supporting solution [75].

3.3.1 Delivery system and imaging function

The functions of delivery agents and imaging are usually combined together for gold nanoparticles being employed for cancer immunotherapy. T cells could be marked by gold nanoparticles as a computer tomography (CT) contrast agent, which could allow to be examined the distribution, migration, and kinetics of T cells. The gold nanoparticles were used to design a composite-based immunostimulatory DNA hydrogel to enhance anti-tumor immune responses by Yata et al. [48]. The loaded hexapod-like structured DNA was released by laser irradiation, which efficiently stimulated immune cells to release proinflammatory cytokines. Then, EG7-OVA tumor-bearing mice, which received the gold nanoparticle hydrogel, were laser irradiated to increase the local temperature and the mRNA expression; the treatment effectively inhibited the growth of tumor and prolonged the survival period of the tumor-bearing mice.

Figure 5: The liposome-based delivery system targeting TAMs.

Meir et al. designed a method for longitudinal and quantitative *in vivo* cell tracking using gold nanoparticles [76]. In the research, T cells were labeled with gold nanoparticles as a CT scanning agent, which were injected intravenously to the mice bearing human melanoma xenografts. The whole-body distribution of the targeted gold nanoparticles was observed through the CT scanning results, and the labeled cells could be clearly seen in the lungs for 48 h after injection (Figure 6). They also compared the gold nanoparticles for CT imaging with fluorescence imaging, and the results indicated that the novel method of cell tracking with gold nanoparticles was an effective tool for research and clinical application for cancer immunotherapy.
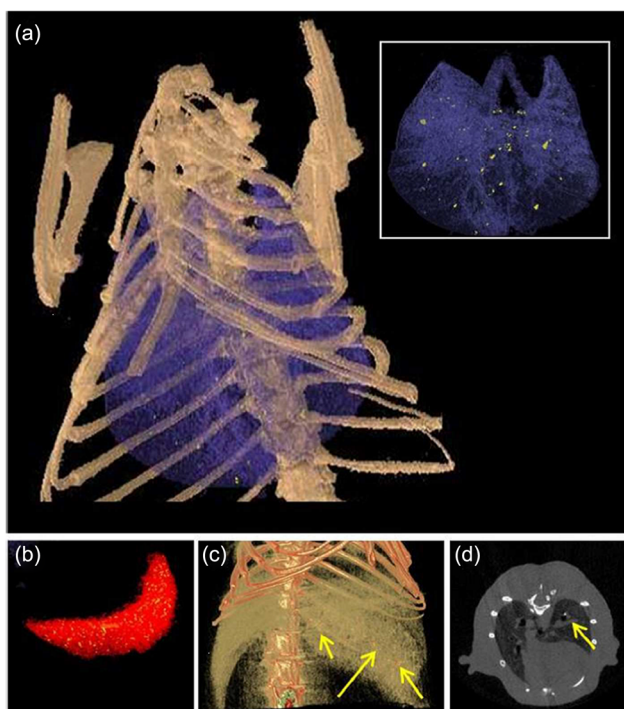
Figure 6: CT scanning of the whole-body distribution and migration of T cells labeled with gold nanoparticles. (a) 3D volume rendering CT image of the labeled T cells, which accumulated in the lungs for 48 h after injection; (b) 3D image of spleen; (c) 3D image of liver; and (d) 2D CT image of lungs.

3.3.2 Targeting Tregs

Gold nanoparticles could also be used for targeting Tregs of TME modulation, and like liposome nanoparticles, gold nanoparticles could mainly target Tregs. Yeste et al. used gold nanoparticles to administer acid methyl ester and a T-cell epitope to promote the generation of Tregs by DCs [77]. The results indicated that the DCs that were treated with gold nanoparticles could promote the differentiation of Tregs *in*

vitro. Furthermore, the gold nanoparticles that were loaded with acid methyl ester and myelin oligodendrocyte glycoprotein peptide fragment (MOG) [35–55] could expand the FoxP3$^+$ Treg compartment and suppress the development of autoimmune encephalomyelitis, and hence, gold nanoparticles were proved to be a novel method for inducing functional Tregs in autoimmune disorders.

3.4 Fe_3O_4 nanoparticles

Fe_3O_4 nanoparticles have been approved by the FDA for clinical use due to their magnetic targeting function and T_2-weighted MRI. But, the individual Fe_3O_4 nanoparticles cannot be used for photothermal therapy because of their low molar extinction coefficient. Fe_3O_4 nanoparticles are prepared by arc discharge, laser ablation, mechanical grinding, microemulsions, nanoprecipitation, high temperature decomposition of organic precursors, etc. [78].

3.4.1 Delivery system and imaging function

Similar to gold nanoparticles, Fe_3O_4 nanoparticles also exhibit the combined functions of delivery system and imaging, which could assist to complete MRI, and the uptake efficiency is highly dependent on the size of the nanoparticles. Ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles with magnetic resonance properties could stay in blood pool for a longer period than large magnetic nanoparticles. In this way, Yuan et al. designed Fe_3O_4-based USPIO nanoparticles, which were subjected to caspase 3 (Casp 3)-instructed aggregation. The results indicated that the Fe_3O_4-based USPIO nanoparticles could enhance T_2 MRI of tumor apoptosis effectively [79]. Moreover, Li et al. synthesized Fe_3O_4 nanoparticles, which were water dispersible, colloidally stable, and biocompatible for imaging and cancer photothermal therapy. The Fe_3O_4 nanoparticles could target specificity to CD44 receptor; additionally, they could be a nanoplatform for MR/CT imaging of cancer cells *in vitro* and xenografted tumor *in vivo*. These results further indicated that Fe_3O_4 nanoparticles could be applied for multifunctional nanoplatform with drug delivery and imaging functions [80].

3.4.2 Targeting immune checkpoint

In the TME modulation, Fe_3O_4 nanoparticles could mainly target the immune checkpoint to deliver the modulators.

A novel Fe_3O_4 nanoparticle-based multifunctional drug carrier was designed and studied by Ge et al. [81]. The carrier was fabricated by (1) mPEG-PLGA, which was applied for encapsulating the spherical super-particles (SPs), (2) imiquimod (R837), which was an immune adjuvant and could promote DCs and phagocytize tumor-associated antigen, and (3) Fe_3O_4 nanoparticles. Thus, the carrier was termed as Fe_3O_4-R837 SPs. The schematic illustration of Fe_3O_4-R837 SPs photothermal therapy with PD-L1 checkpoint blockade for cancer immunotherapy is shown in Figure 7. The carrier could destroy the tumors with NIR irradiation, and the antigens could induce strong antitumor immune responses. The method in combination with PD-L1 checkpoint blockade was proved to eliminate primary tumors, prevent metastasis, and inhibit the growth of tumor cells. The results further indicated that Fe_3O_4-based nanoparticles could be applied for cancer immunotherapy by targeting immune checkpoint in TME modulation.

3.5 Carbon nanotube

Carbon nanotubes are widely used for cancer immunotherapy due to their excellent properties of drug delivery, non-toxicity, nonimmunogenic, and diagnostics.

In the recent research studies and applications of multifunctional carbon nanotubes, they are mostly used as a drug delivery system for antigens, adjuvants or gene, and adjuvants themselves. Carbon nanotubes were fabricated by arc discharge, template methods, chemical vapor deposition, electrolysis, flame synthesis, electron or ion beam irradiation, laser ablation, pyrolysis, solar approaches, etc. [82].

3.5.1 Delivery system and self-adjuvant

When carbon nanotubes deliver the agents to generate immune response, they could also act as self-adjuvants to generate the "danger signals" to promote the antigens presentation and immune responses, but the cytotoxicity becomes the limitation. Parra et al. assessed the immune response–amplifying properties of carbon nanotubes to haptens with azoxystrobin. Four kinds of functionalized CNT–BSA–AZc6 nanotubes in different sizes, which were 1–2 nm in diameter with 5–30 μm in length and 50–80 nm in diameter with 10–20 μm in length, were linked to BSA–AZc6 conjugate, which was fabricated with an azoxystrobin derivative bearing a carboxylated spacer arm (hapten AZc6) and bovine serum albumin (BSA). The results indicated that the IgG-type antibody responses were assessed in terms of the titer and affinity.
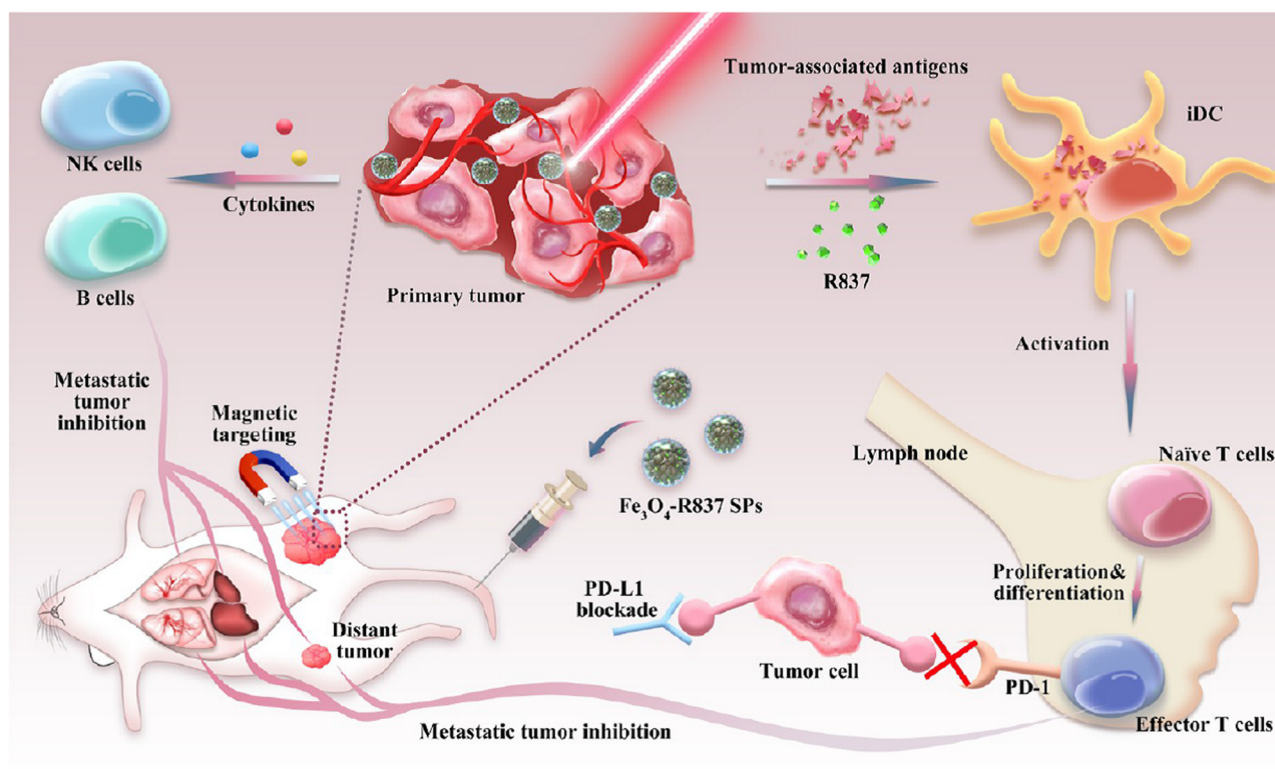
Figure 7: The schematic illustration of Fe_3O_4-R837 SPs photothermal therapy with PD-L1 checkpoint blockade for cancer immunotherapy.

Moreover, the CNT–BSA–AZc6 nanotubes could act as an adjuvant themselves to obtain IgG responses without any other additional adjuvants. They also found that the shorter immunogens would generate the stronger responses due to the better uptake by APCs when carbon nanotubes were less than 1 μm in length [28].

Jambhrunkar et al. developed pristine mesoporous carbon hollow spheres as protein carriers and adjuvants for generating the immune responses [83]. Invaginated mesostructured hollow carbon spheres (IMHCSs) have excellent properties, including high loading capacity, controllable ovalbumin release, safety profile, and high antigen delivery efficacy. They indicated that the novel designed IMHCSs were safer adjuvants than QuilA, which could better promote Th2-biased immune responses for nano-vaccine delivery.

3.5.2 Gene delivery

Relatively large size DNA, mRNA, shRNA, etc., could also be encapsulated with carbon nanotubes to be protected and delivered. Taghavi et al. designed a novel carbon nanotube-based delivery system for shRNA delivery,

which was fabricated by a modified branched polyethylenimine (10 kDa), polyethylene glycol (PEG), single-walled carbon nanotubes (SWCNT), AS1411 aptamer, and a very low DOX content. The schematic illustration of shRNA and DOX co-delivery into gastric cancer cells through the delivery system is shown in Figure 8. The results indicated that the combination treatment of cancer therapy using the novel delivery system could inhibit the growth of gastric cancer cells and further revealed that the carbon nanotube-based delivery system could be applied for antitumor activity [84].

3.5.3 Targeting Tregs

Carbon nanotubes could also modulate TME by targeting Tregs. Sacchetti et al. were the first to focus on intratumoral immune cell targeting investigation to explore the ability of ligands against Treg-specific receptors to drive selective internalization of PEG-modified single-walled carbon nanotubes (PEG-SWCNTs) into Treg residing in the tumor microenvironment. The results indicated that Tregs targeting efficiency was dependent on incubation time, dose,
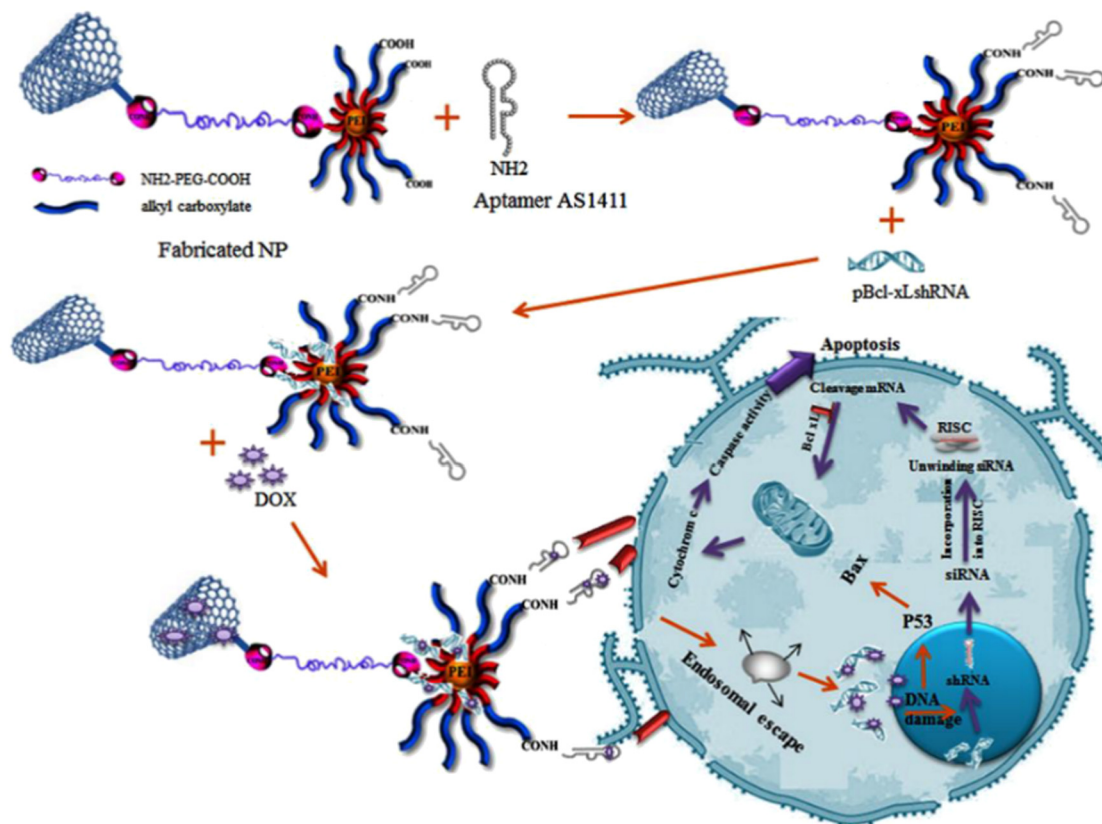
Figure 8: The schematic illustration of shRNA and DOX co-delivery into gastric cancer cells through the delivery system.

number of ligands, and surface marker; moreover, the PEG-SWCNTs conjugated with anti-GITR antibody (DTA-1) against glucocorticoid-induced TNFR-related receptor (GITR) could be internalized through receptor-mediated endocytosis by intratumoral Tregs [85].

3.6 Porous silicon

Porous silicon nanomaterials have drawn a great attention for drug delivery system and self-adjuvant in cancer therapies due to their properties of controlled geometry, tunable nanoporous structure, high specific surface area, and versatile surface chemistry. The porous silicon-based delivery system could deliver the antigens to DCs directly, prolong the release, be functionalized with adjuvant molecules, and increase the cross-presentation of antigens. But similar to carbon nanotube, porous silicon is non-biodegradable and long-term cytotoxic. Similar to PLGA nanoparticles, the synthesis of porous silicon could also be divided into two parts: "bottom up" method and "top down" method. The "bottom up" method starts with a porous template, then the template is filled up and etched to obtain porous silicon nanoparticles. The "top down" method starts with bulk sized silicon, followed by electroless etching, electrochemical etching, etc. [86].

Fontana et al. designed three porous silicon-based nano-vaccines for cancer immunotherapy, including thermally oxidized porous silicon (TOPSi) encapsulated into acetalated dextran (AcDEX) (TOPSi@AcDEX), TOPSi encapsulated into spermine-modified AcDEX (SpAcDEX) (TOPSi@SpAcDEX), and TOPSi encapsulated into AcDEX and cancer cell membrane (CCM) (TOPSi@AcDEX@CCM). These results indicated that TOPSi@AcDEX could be encapsulated with vesicles derived from cancer cells to combine with the adjuvant properties of porous silicon; TOPSi@SpAcDEX could induce the expression of co-stimulatory signals in the immortal cell lines and blood monocytes; TOPSi@AcDEX@CCM could enhance the secretion of IFN-γ in peripheral blood mononuclear cell (PBMC) without inducing the secretion of IL-4. Overall, the three porous silicon-based nano-vaccines could be an alternative delivery system with promising adjuvant properties for the materials derived from patient's tumor [87].

Mahony et al. investigated the immunization to the OVA by using mesoporous silica nanoparticle-based delivery system and self-adjuvant in mice. The research indicated that the amino-functionalized MCM-41 nanoparticles could act as a self-adjuvant and elicit immune responses without local or systemic side effects [88].

3.7 Iron dextran and quantum dots as aAPCs

Iron dextran and quantum dots are basically functionalized with aAPCs only in cancer immunotherapy. For quantum dots, there are mainly two approaches for the preparation: (1) formation of semiconductor nanoparticles through colloidal chemistry and (2) epitaxial growth and patterning in nanoscale [89].

Iron dextran nanoparticles are mainly used as aAPCs in cancer immunotherapy due to their extensive characterization and biocompatibility. Perica et al. synthesized iron dextran-based aAPCs to rapidly expand tumor-specific T cells by "enrichment" and "expansion" methods. The schematic of synthesis is shown in Figure 9. First, the iron dextran nanoparticles that were 50–100 nm in diameter were synthesized by coupling MHC-Ig dimer and CD28 antibodies. Then, the antigen-specific CD8+ T cells were bound to iron dextran aAPCs and proliferated in "enrichment" and "expansion" steps, respectively. The results indicated that strong T-cell responses were generated for both shared tumor antigens and computationally predicted neo-epitopes. The study further reported that the iron dextran-based aAPCs synthesized by "enrichment" and "expansion" could be an effective treatment for cancer immunotherapy [90].

Perica et al. also manufactured and compared two types of aAPCs fabricated by iron dextran paramagnetic particles, which were 50–100 nm in diameter, and avidin-coated quantum dot nanocrystals, which were around 30 nm. They reported that both iron dextran particles and quantum dot nanocrystals could enhance tumor retention in a subcutaneous mouse melanoma model, leading to effective T-cell stimulation and inhibition of tumor growth *in vivo* [57].

4 Conclusions and perspectives

In this review, we summarized and discussed the various nanomaterials, including PLGA nanomaterials, liposome nanoparticles, gold nanoparticles, Fe_3O_4 nanoparticles, carbon nanotubes, porous silicon-based nanomaterials, iron dextran, and quantum dots, with different functions and properties applied for cancer immunotherapy. Nanomaterials could overcome the limitations of conventional delivery systems and provide an excellent breadth of novel methods to generate specific immune responses for cancer immunotherapy. Nanomaterial-based immunotherapy could produce long-lasting and

(a)

(b)
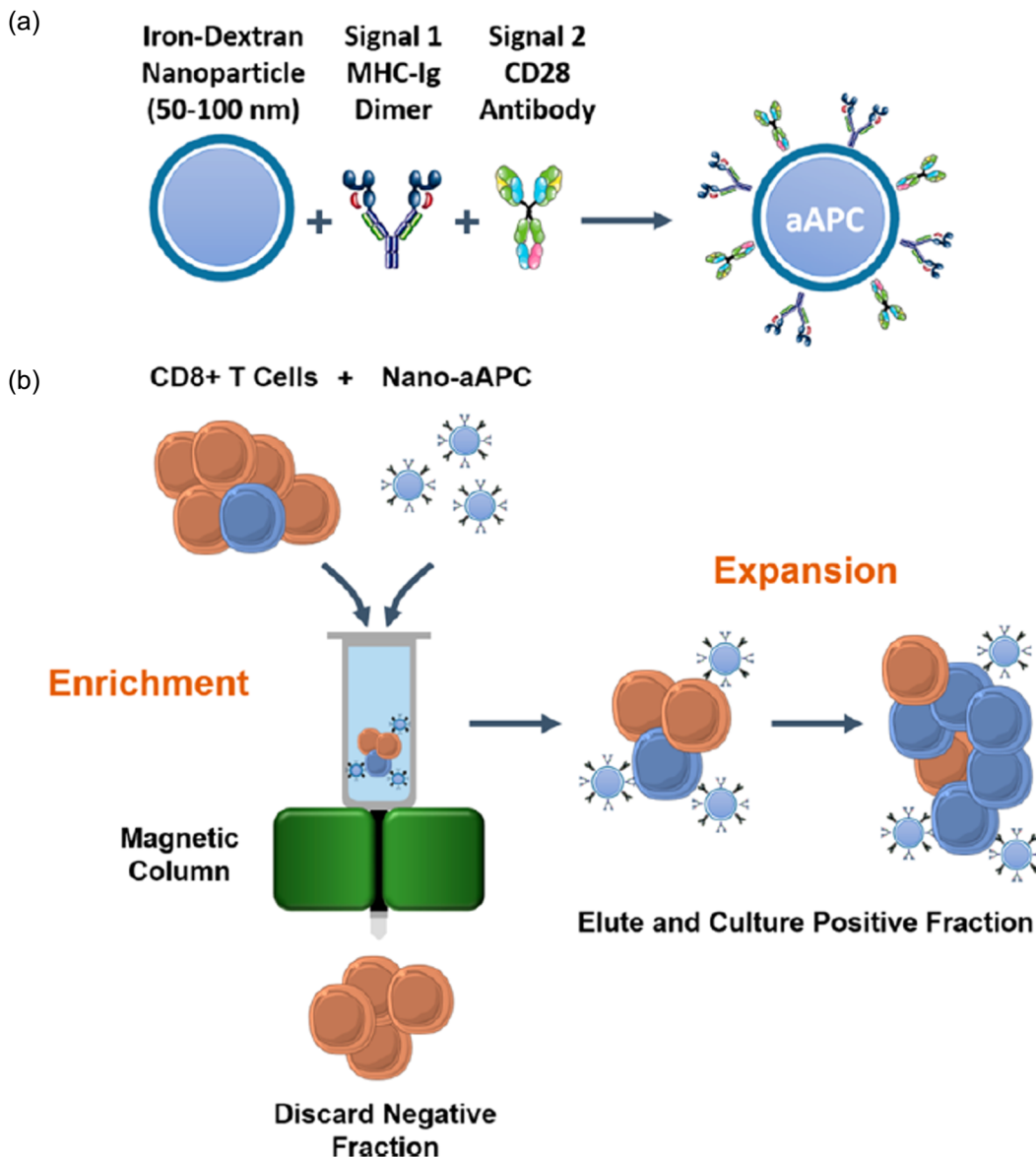
Figure 9: The schematic of synthesis of iron dextran-based aAPCs.

broader immune responses through the functions of delivery and co-delivery of antigens, adjuvants, genes, peptides, and whole cell antigens to APCs; self-adjuvant; and targeting TME modulation. Nanomaterials play a more important role in cancer immunotherapy, and cancer immunotherapy will be further developed by using the novel nanomaterials. Ultimately, the research and development will benefit the patients suffering from cancer.

Despite nanomaterials have a lot of advantages for cancer immunotherapy, there are still some disadvantages and problems needed to be further investigated. First, the safety and the systemic cytotoxicity caused by nanoparticles from cellular level to animal level are still not clarified enough. Second, even the nanomaterials could generally increase the efficiency of antigen delivery, DCs uptake, antigen presentation, or TME modulation, it is still not clear enough that which nanoparticle is more effective with less side effects for specific tumors like lung cancer or breast cancer. The interactions between nanomaterials and immune system are still needed to be studied and clarified. Third, the biggest advantage of nanoparticle is the small size, which is in nanoscale. However, the effects of nanoparticle size on the functions and efficiency are still needed to be studied. Besides, if the nanomaterial in particular size could be commercially manufactured still needs to be investigated. Last but not least, the relatively simple

function equipped with nanoparticles exhibits low efficiency for cancer immunotherapy.

In future research, the author considers four aspects as the research focuses. (1) Developing the novel nanomaterials that are safer to use and have low toxicity from cellular level to animal level and high specificity. Sometimes, the immunotherapy with nanomaterials could fail to generate robust immune response or the generated immune response is irrelevant for the particular tumor or patient. So, the novel nanomaterials which could achieve the right signals to generate the specific immune response for the specific tumor or patient should be focused on. (2) The nanomaterials could be designed with multifunctions individually for specific patient. For example, we can try to combine two or more nanoparticles to supply each other to obtain the composite nanoparticles in reasonable size with comprehensive specific functions for the specific conditions and patients, (3) Immunodeficient mice xenografted with human cancer cell lines are normally used for *in vivo* cancer research, but they are not suitable for studying cancer immunotherapy. So, the further research studies should employ novel experiment methods, or evaluation criterion, which could connect the results of animal experiment with real human reactions, or even new animal model to better mimic pathophysiological conditions of humans. (4) Cancer immunotherapy is a super complicated science and engineering combined with oncology, immunology, material science and engineering, etc. The application of nanomaterials probably could not solve all problems and limitations during the treatment. The well-controllable artificial robots in macro- or nanoscale have the potential to fill the vacancy. For the next-generation cancer treatment, we believe that it is possible that the artificial robots encapsulated with specific antigens and adjuvants are perfectly controlled to deliver them to DCs or participate in antigen presentation to generate a robust immune response or even can participate in other steps of immune cycle for cancer treatment.

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