Trends in Immunotherapy (2019) Volume 3 Issue 2, pp.79–88.
doi: 10.24294/ti.v3.i2.95

REVIEW ARTICLE

Role of targeting nanoparticles for cancer immunotherapy and imaging

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ABSTRACT

Cancer immunotherapy involves the delivery of immunogenic compounds and/or the priming, or induction, of the body's natural immune system to target cancer. The use of cancer immunotherapy has led to various means of cancer prevention and treatment that have produced prolonged life expectancy and stabilized disease. Nanoparticles are promising vehicles or adjuvants for effective delivery of therapeutics, antigens, stimulatory effectors, or antibodies for therapeutic invention. Targeting nanoparticles are especially useful due to their capability of accumulating in specific sites of interest like tumors and, thereby, decreasing risks of damage to normal tissue. Targeting can be achieved by incorporation of cell-surface related binding molecules or antibodies. This review explores the role of targeting nanoparticles as delivery or adjuvant systems to modulate immune response, and as imaging tracking systems for cancer immunotherapy.

***Keywords**: nanoparticles; cancer immunotherapy; imaging; targeting*

ARTICLE INFO
Received: July 11, 2019
Accepted: August 7, 2019
Available online: August 18, 2019

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CITATION
Wen R, Umeano AC. Role of targeting nanoparticles for cancer immunotherapy and imaging. Trends Immunother 2019; 3(2): 79–88. doi: 10.24294/ti.v3.i2.95.

Introduction

Surgery, radiation, chemotherapy and their combinations are well established standards for treating cancer, one of the most life-threating diseases worldwide[1]. Metastatic spreading of cancer cells, however, inspires the development of new strategies towards eradicating cancer cells from normal tissue. Cancer immunotherapy uses the body's natural immune systems as a defense for fighting existing cancer or preventing potential cancer(s). Various uses of cancer immunotherapy have provided promising strategies towards achieving long-lasting and effective cancer therapy.

The body is equipped with a natural defense against cancers, viruses, and bacterial pathogens. The primary immune system responsible for cancer immunotherapy is the adaptive immune system. The adaptive immune system is comprised of two major responses: (1) humoral-mediated response and (2) cell-mediated response. Both processes occur when an antigen of interest is phagocytosed by an antigen presenting cell (APC). APCs process phagocytosed antigens and present them on their cellular surface using major histocompatibility complex (MHC) class II. Once a presented antigen is recognized through binding a specific T-cell population, one of the two processes occurs. The process of humoral-mediated or antibody-mediated response, results in the mass production of antibodies by B-cells, activated by T-cells, which bind and target pathogenic bacteria for phagocytosis by macrophages and neutrophils and is often not the employed or desired response for cancer immunotherapy. Cell-mediated immune response occurs through cytokine activation of T-cells, which target and destroy affected/infected cells that present dysregulated cellular or viral proteins through MHC class I receptors. The overview of both processes is outlined in **Figure 1**. Cancer cells mask themselves from the body's natural immune defense in various ways. However, recent technologies have been developed to re-prime the body's existing cell-mediated immune response to destroy these cancer cells.

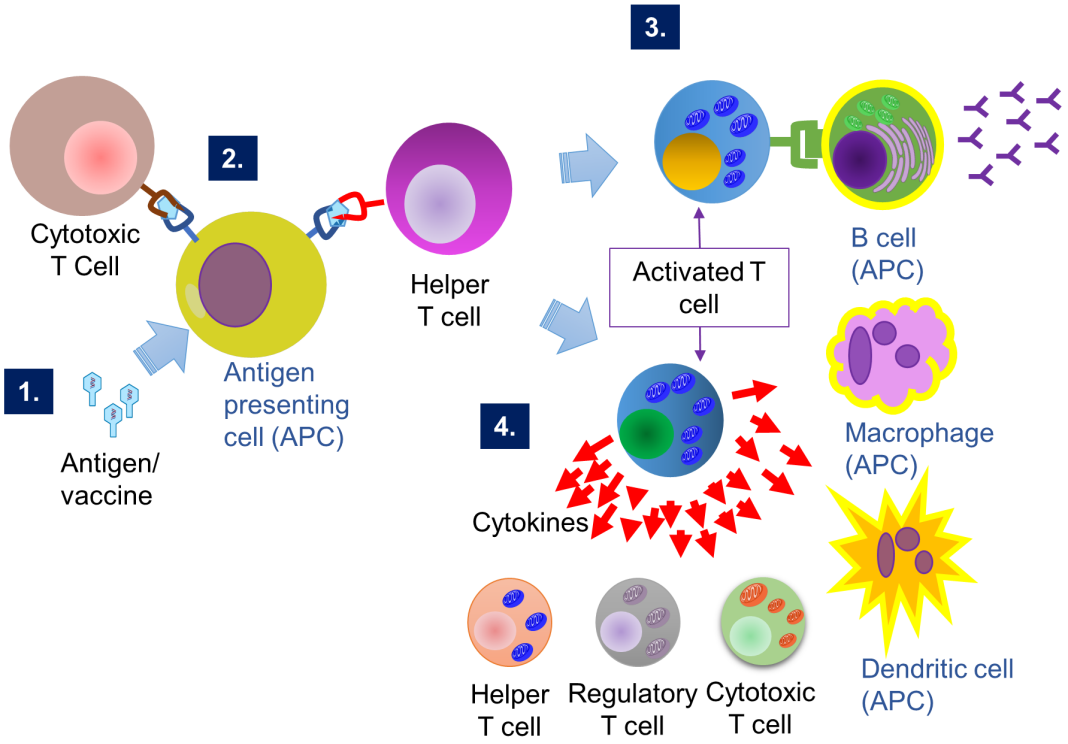

Figure 1. Humoral- and cell-mediated immune response to antigen stimulation. (1) Antigen is phagocytosed by antigen presenting cells (APCs), such as dendritic cells, macrophages/monocytes and B cells. (2) APCs present processed antigen via major histocompatibility complex (MHC) class II receptors to T cells. (3) Helper T-cells stimulate B-cell mediated antibody production in humoral immune response. (4) Cytotoxic and helper T-cells promote cytokine activation of effector cell populations for cell-mediated immune response, or MHC class I-mediated destruction of affected cells.

Several clinical trials have demonstrated the success of cancer immunotherapy, such as immune checkpoint therapy using blocking antibodies to cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed death-1 (PD-1), and chimeric antigen receptor (CAR) therapy, for priming the immune system[2]. Human papillomavirus (HPV) and hepatitis B vaccine have been available for routine vaccination and have improved overall cancer prevention[3–5]. Initial clinical trials on the combination of immunotherapy and radiation in various tumor sites show better localization control, decreased metastatic spreading, and improved overall survival rate[6].

Through the incorporation of nanotechnology, nanoparticles are used as vehicles or adjuvants to effectively deliver imaging agents, therapeutics, antigens, stimulatory effectors, or antibodies to sites of tumor for immunotherapy. Various nanoparticles have been constructed as delivery systems, such as liposomes, quantum dots (QDs), nano-dendrimers, nano-micelles, nano-hydrogels, polymer-based, carbon-based, and inorganic metal or metal oxide based nanoparticles. Introducing immunotherapies with targeted treatments at the molecular level is of particular interest and can be a promising strategy for effective cancer therapy. This review discusses the targeted nanoparticle-based intervention for immune modulation as carrier or adjuvant system as well as its application in immune system tracking as imaging agents or delivery systems.

Targeting nanoparticles for immunotherapy

Rapid progress has been made in the application of nanotechnology to various diseases, either as drug or imaging agent delivery system, or as adjuvant therapeutic agents. Targeting control is essential to reduce side effects associated with off-target distribution and enhance therapeutic intervention when vaccine is administrated in clinical settings. The exclusive delivery to specific cell types or tissues by nanotechnology is of great interest and promising for effective therapeutic intervention. The properties of nanoparticles can be modified to control their distribution behavior by various ways, wherein a specific targeting ligand can be attached *via* surface modification, conjugating or as part of the nanoparticle components. Nanoparticle delivery system can be constructed by various formulations,

such as liposome[7], polymers[8], micelle[9], or lipid-polymer hybrid[10]. The targeting of the adaptive immune system plays a significant role in effective vaccine or immunotherapy, in which antigen is largely or exclusively enriched in the specific sites inducing immunomodulatory effectors in lymphoid environment[11]. Possible cellular targets for nanoparticles are illustrated in **Figure 2**.

APCs consist of professional APCs such as dendritic cells (DCs) expressing antigens and MHC class II molecules, and non-professional APCs expressing MHC class I molecules such as fibroblast and endothelial cells[12–14]. APCs can present antigens to T cells for immune response through binding with MHC class I molecules for T cells expressing CD8 cells, and MHC class II molecules for CD4 cells[15]. The APCs mainly include DCs, macrophages and B cells. DCs are mostly studied as targets for immunotherapy[16], other types of APCs such as astrocytes may be used as targets for brain tumor therapy. Therefore, APCs are possible targets to nanoparticle-based delivery system for stimulating efficient immune response for cancer therapy.

Dendritic cells

DC is one of the most potent APCs for initiating and modulating immune response. DCs have been used as targets for antigen-loaded nanoparticle system in most studies. Size control is a key regulator in targeting nanoparticle system for DCs. An *in vivo* mice model indicates that large (1000–2000 nm) polystyrene particles were predominantly detected

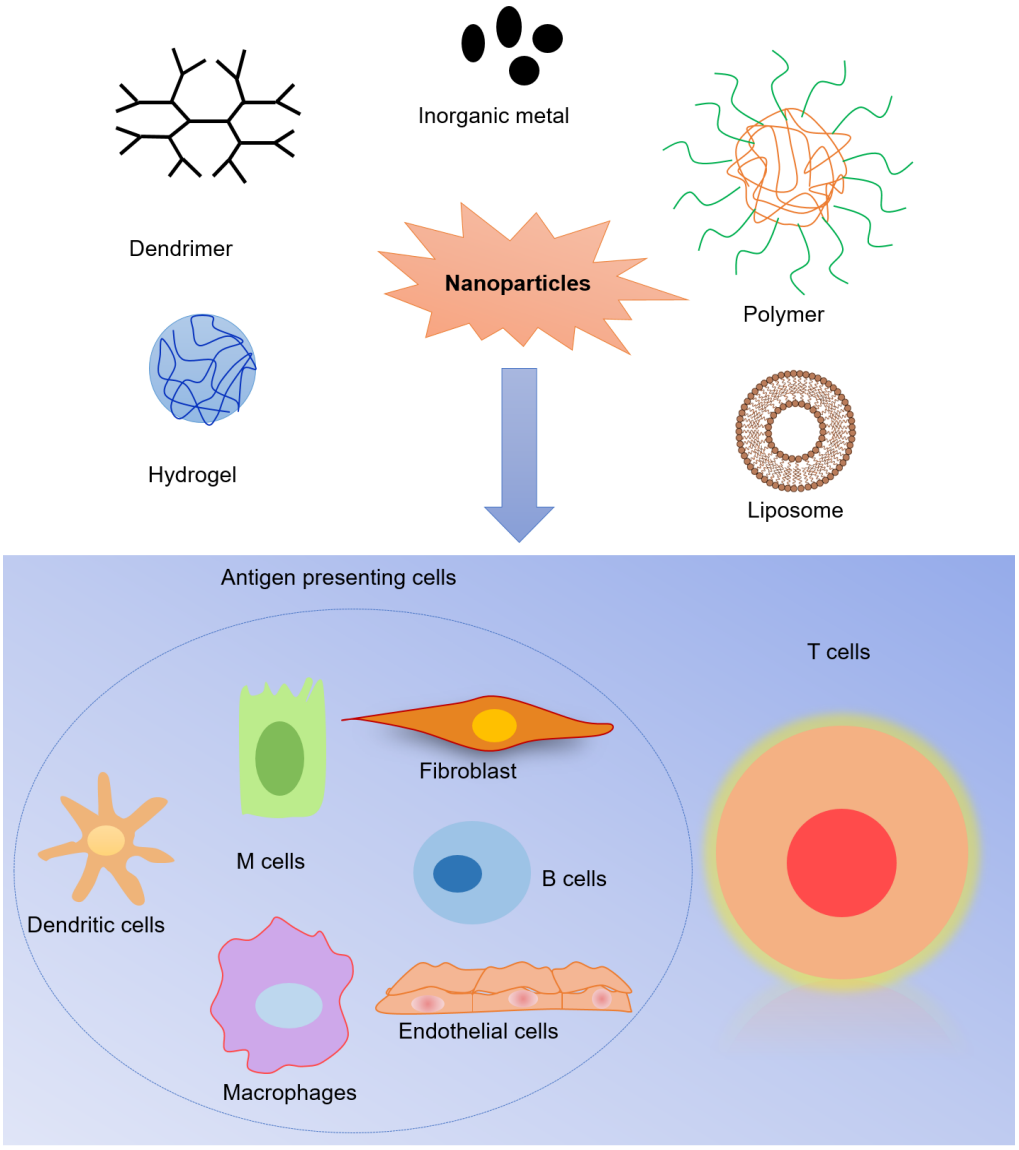

Figure 2. Potential cell level targets for nanoparticle-based immunotherapy

in DC and small (20–200 nm) nanoparticles were mainly located in lymph-nodes resident DCs[17]. Poly(ethylene glycol)-stabilized poly(propylene sulfide) (PPS) nanoparticles are reported to reside in ~50% of DCs in the lymph nodes without a targeting ligand. The small size range of 20–45 nm PPS nanoparticles was suitable for both uptake and retention in lymph node, and 100-nm PPS nanoparticles were not found in lymphatic site[18]. Poly(γ-glutamic acid) (PGA) nanoparticles demonstrate effective internalization in DCs when injected in mice and induced the generation of interleukin (IL)-12p40 and IL-6. The ovalbumin (OVA)-loaded PGA nanoparticles showed significant levels of interferon (IFN)-γ, antigen specific immunoglobulin G (IgG), IgG1, and IgG2a antibodies and more potent cytotoxic T lymphocyte (CTL) response than free OVA in a 7-day immunization study. The incorporation of listeriolysin O (LLO) antigen in PGA nanoparticles significantly enhanced the survival rate of mice in a fourteen-day immunization for lethal *Listeria monocytogenes* prevention[19].

It is possible to effectively target antigen-nanoparticles to DC *via* cell-surface molecules to enhance strong immune potency for cancer therapy. A targeting ligand such as an antibody is commonly used to achieve the goal of nanoparticles targeting. A humanized targeting antibody hD1 is reported to target DCs *via* interaction with human C-type lectin receptor DC-SIGN. Poly(lactic-co-glycolic acid)-based nanoparticles (PLGA-NPs) could be effectively delivered to DCs when incorporating hD1 as targeting moiety[20]. The DEC-205 antibody was used as DC targeting ligand as well for nanoparticles 200–250 nm in diameter. DEC-205-OVA PLGA-NPs showed similar level of IFN-γ and IL-2 production, but significantly more of IL-5 secretion than non-targeted nanoparticles, and a production of IL-10 cytokine *in vitro*. *In vivo* model confirmed that DEC-205-OVA CFA nanoparticles could induce T cell production of IL-10 and IL-5, with more cytokine generated upon greater density of anti-DEC-205[21]. The PLGA-NPs could also be selectively internalized to DCs when using α-CD40-mAb targeting moiety. The multi-compound loaded PLGA-NPs composed of a protein antigen (*e.g.* OVA), Pam3CSK4 and Poly(I:C) and α-CD40-mAb showed highly efficient delivery to DC in the *in vivo* model when injected in mice, stimulated significantly boosted $CD8^+$ T cell responses, and prolonged the survival rate in vaccinated mice compared to non-treated and non-targeted nanoparticle control[22]. A comparative study of targeting various DC cell-surface molecules indicated that CD40, DEC-205 or CD11c targeted nanoparticle showed similar capability to induce $CD8^+$ T cell immune response[23].

Active targeting of antigens in DCs by nanoparticles can also be achieved by other targeting ligands, such as mannose or lactose residues, *via* endocytic receptor interaction to elicit robust vaccines[24]. Mannan (MN)-decorated PLGA-NPs showed an increased cellular uptake in DCs by both flow cytometry and confocal microscope[25]. Fc fragment of human IgG is used as an efficient targeting system *via* Fc receptor interaction for gold nanoparticles (AuNPs) and liposomes for superior intracellular uptake and antigen presentation in DCs to that of free peptide control[26]. Surface-functionalized mannose polyanhydride nanoparticles are reported to effectively activate DCs through mannose receptor. The dimannose-modified nanoparticles demonstrated the improved MHC II, CD86 and CD40 antigen presentation, increased CD206 and *CIRE* expression, higher level of pro-inflammatory IL-6 and TNF-α compared to non-modified nanoparticles[24]. Asparagine-glycine-arginine (NGR) cell-specific peptide is reported as a targeting ligand to target PEGylated polyplex to DC *via* enhanced phagocytosis. The NGR peptide-PLGA-PEG-PLGA nanoparticles showed non-toxicity to DCs at an optimal concentration of 0.25% of copolymer[27].

Subcellular compartments are potential targets for nanoparticles to improve antigen presentation and potentially induce robust immune responses[28]. The pH-sensitive nanoparticles have been developed to directly disrupt endosomes and/or lysosomes in response to acid environment to release antigens into cytosol. Nanoparticles composed of hydrophobic pH-responsive tertiary amines core and hydrophilic shell 2-aminoethyl methacrylate (AEMA) cross-linked by poly(ethylene glycol) methacrylate (PEGDMA) showed effective delivery of OVA antigen to cytosol of DCs, and 4-fold increasing of IFN-γ production from T cells than pH-insensitive and free OVA controls[29]. Cationic acid-degradable nanoparticles are reported to direct improved OVA antigens presentation *via* MHC class I pathway in response to acidic lysosomes. The cationic nature elicited nanoparticles to be endocytosed or phagocytosed into bone marrow-derived DCs (BMDCs) at the initial stage of cellular uptake. The CpG-oligodeoxynucleotides (ODN)-coated acid-degradable nanoparticles produced 10 more fold of IL-12 than free CpG ODN, or nanoparticles without CpG-ODN in BMDCs[30].

Endoplasmic reticulum (ER) is an alternative subcellular target for immunotherapy. ER-targeting

can be achieved by a peptide or sequence containing ER targeting moieties like KKXX signal. ER-targeting peptide is reported to intracellular traffic PLGA-NPs to ER. This targeting peptide-conjugated nanoparticles induced low efficiency of SIINFEKL cross-presentation at initial time (2 h) and prolonged cross-presentation of SIINFEKL up to 48 h *in vitro*[31]. Intensive *in vitro* and *in vivo* studies of this nanoparticle system are not yet reported. Gelatin nanoparticles are reported to target tetramethylrhodamine-dextran (TMR-dextran) to lysosomes of DCs[32]. Cationic gelation nanoparticles loaded with CpG ODN strongly inducted stimulatory effectors for immune response within DCs both *in vitro* and *in vivo*[33]. Efficient gene targeting can be realized from endosomal escape by using a nanoparticle system for cancer immunotherapy. Octa-arginine (R8)-modified lipid nanoparticles are reported to deliver short interfering RNA (siRNA) to DCs for gene knockdown. R8 modification facilitates cellular uptake of nanoparticles *via* macropinocytosis to avoid lysosomal degradation. The siRNA-loaded R8-modified nanoparticles efficiently reduced the endogenous *SOCS1* gene expression and showed enhanced *in vivo* vaccine potential with suppressed tumor growth after immunization compared to the control BMDCs[34].

Macrophages

Macrophages are possible targets in stimulating immune response and/or tolerance as well for nanoparticle-based cancer vaccine and/or therapy. Macrophages express various surface molecules such as Fc receptors, Toll-like receptors (TLRs), integrin, glycosylphosphatidylinositol (GPI)-anchored, mannosyl, and galactosyl receptors[35,36]. One approach is to incorporate targeting ligands to nanoparticles for efficient cellular uptake *via* binding with macrophage surface receptors. Similar to DCs, polyanhydride nanoparticles were also used for macrophage targeting when conjugated with dimannose and galactose *via* C-type lectin receptors. The dimannose functionalized nanoparticles induced significantly higher levels of macrophage mannose receptor, while galactose-modified nanoparticles expressed more macrophage galactose lectin (MGL). The functionalized nanoparticles significantly enhanced the expression of CD40 and cytokine production of cytokines IL-1β, IL-6 and TNF-α by alveolar macrophages[37]. Liposomes are mostly used for various drug delivery to macrophage, ligands such as antibody L-Ab is able to modify liposomes for delivery of various materials such as dideoxycytidine triphosphate (ddCTP) to human macrophages *via* Fc receptor mediated pathway[38]. These systems may be used as antigen carriers or adjuvants for macrophage-related tumor vaccine.

Microfold cells

Microfold cells (M cells) are a type of epithelial non-APCs lacking rigid cytoskeleton and presenting broad membrane microdomains on apical surfaces. The antigen-loaded system firstly undergoes endocytic or phagocytic uptake by M cells and antigens are rapidly directed to the intraepithelial pocket containing immune cells like B and T lymphocytes *via* transcytosis for immune responses[39]. The density of M cells is usually very low in the gastrointestinal tract, hence, specific targeting of M cells by nanoparticle delivery system is attractive for robust immune responses. Fievez *et al*. reported arginine-glycine-aspartate (RGD)-grafted PLGA-PEG nanoparticles (RGD-NPs) as oral vaccine system for M-cell targeting through selectively interacting with integrin. OVA-loaded RGD-NPs elicited immune response with significantly higher level of IgG antibodies and IFN-γ *in vivo* intragastric administration[40]. Surface receptors related to M cell endocytosis or phagocytosis can be used as targets for antigen-nanoparticle system. Claudin 4 is such a receptor that highly expressed in M cells. C-terminal targeting peptide, CPE30 is reported as targeting ligand to bind Claudin 4 in PLGA-NPs system. HA-HT-CPE30 nanoparticles showed effective uptake by M cells both *in vitro* and *in vivo* as targeted mucosal vaccines[41].

T lymphocytes

T lymphocytes possess various antigen-specific receptors, such as Ti and T3[42], which can be served as potential targets for antigen-nanoparticles to elicit T-cell immune responses. The binding of CTLA-4 with CD80 or CD86 to inhibits the T-cell activation and blockage of CTLA-4 can be used to enhance T-cell activation and memory[43]. The combination of CTLA-4 with other vaccines such as GM-CSF and PD-1 can significantly suppress the tumor progress *via* modulating autoimmune[44,45]. Tumor associated antigens (TAAs) can be directly carried to T cells for promoting immune secretory activation. PLGA-NPs are reported to load tyrosinase-related protein 2 (TRP2) as TAA and 7-acyl lipid A as TLR4 to induce robust $CD8^+$ T cell-activated anti-tumor immunity *in vivo*[46]. Cui *et al*. reported human immunodeficiency virus type 1 (HIV-1) vaccine by delivering Tat (1–72) antigen in a nanoparticle system to promote Tat-specific T cell proliferation for robust immune response *via* generating antibodies and cytokines[47].

Imaging role of targeted nanoparticles

Imaging tracking of immune cells is important in understanding the latent mechanisms of immune cell-based therapy in clinical practice. Nanoparticle system is a possible strategy for noninvasively monitoring immune cell trafficking in a longitudinal fashion. Nanoparticles can be used as delivery systems incorporating imaging agents or as agents themselves for cell/tissue monitoring.

Fluorescent-based labeling

The most commonly used agent in cell tracking is fluorescent or bioluminescent-based probe, in which reporter genes expressing fluorescent protein, fluorescent or bioluminescent probe are used for labeling. Fluorescent nanoparticles for immune cells imaging can be made with fluorochromes or gene expressing fluorescent protein loading in polymeric system, inorganic metal oxide such as silica nanoparticle and liposomes. The reporter gene is superior to fluorescent-based probe as it lasts as long as a cell lives by continuously expressing optimal-based protein for imaging. However, the fluorescent- or bioluminescent-based methods have low tissue penetration. Yu *et al*. reported TopFluor-labeled cholesterol as lipid component for constructing virus-like nanoparticles which were surface modified by CD169, which was used as nanoparticles tracking for uptake behavior in DCs[48]. Near-infrared (NIR) fluorochromes (700–1000 nm) are widely used in imaging system as it penetrates deep tissues[49].

In addition, the nanoparticles alone can also be used as fluorescent probes, such as QDs, carbon dots (CDs) and upconversion nanoparticles (UCNPs), which are good alternatives to fluorescent dyes due to high resistance to photobleaching, deep penetration, high physicochemical and photochemical stability[50–52]. OVA-loaded UCNPs (OVA-UCNPs) are used to stimulate DCs for immune secretory as well as *in vitro* and *in vivo* precise nanoparticle tracking[53]. UCNPs can conduct photodynamic therapy (PDT) as well as be used as imaging agents in cancer immunotherapy. The unique NIR-to-visible upconversion process can be used to activate photosensitive therapeutic agents such as ZnPc[54] for TAAs production to stimulate immune secretory in cancer vaccine. A recent study incorporated a photosensitizer Ce6 and TLR-7 agonist R837 into UCNPs (Ce6-R837-UCNPs). The constructed Ce6-R837-UCNPs were able to penetrate to deep tumor tissue under NIR photoirradiation and caused TAAs generation promoting strong immune response *in vivo*. The UCNPs showed good NPs labeling efficiency *in vitro* and this may also be detected *in vivo* before conducing PDT treatment[55].

Magnetic resonance imaging contrast agent-based labeling

Magnetic resonance imaging (MRI) technique utilizes magnetic field to visualize organs and structures of body system[56]. Inorganic paramagnetic metal or metal oxide is used for MRI, such as iron oxide and gadolinium-based nanoparticles[57,58]. The contrast agents-loaded nanoparticle system effectively enhances the cellular uptake by modulating the size and surface modification with targeting ligand in cancer immunotherapy. Fe_3O_4-ZnO core-shell nanoparticles (Fe_3O_4-ZnO-NPs) were reported to play dual roles in cancer immunotherapy by delivering carcinoembryonic antigen into DCs and simultaneously being used as an MRI agent. The Fe_3O_4-ZnO-NPs were effectively internalized into DCs, which were used for immunization *in vivo* and showed better tumor growth inhibition and prolonged survival than controls[59]. Besides, molecule probe that contains ^{19}F such as perfluorocarbon or induces chemical exchange saturation transfer can be possible MRI contrast agent in biomedical imaging[56]. PLGA-perfluorocarbon nanoparticle is reported to distinct plasmacytoid and myeloid DCs by ^{19}F MRI in clinical settings[60].

Computed tomography contrast agent-based labeling

Computed tomography (CT) is X-ray based imaging technique widely used in biomedical field[61]. CT contrast agents are used for tracking behavior to evaluate the recruitment of immune cells and monitor the cellular immunotherapies. Nanoparticle is a possible system to deliver CT contrast agents for imaging since attributed to long retention time and fine-tuned size control. Many nanoparticle carriers are used in medical imaging, including liposomes, micelles, lipoproteins, polymeric nanoparticles, inorganic metal nanoparticles, silica and carbon nanotubes[62], these nanoparticles can be applied as imaging vesicles for immune cells as well. A cell-specific targeting ligand would facilitate the localization of nanoparticles in immune cells of interested. Inorganic metal nanoparticles are potential CT contrast agents, which includes AuNPs, iodine-based and bismuth-based nanoparticles[63]. AuNPs of 20 nm in diameter are reported as CT agents to *in vivo* image cancer-specific T cells. The Au labeling was not toxic and did not affect T cell function at a concentration less than 0.75 mg/mL *in vitro*. The targeted T-cells labeled with AuNPs for CT showed increased accumulation of T-cells in tumor

sites than non-targeted control when intravenously administrated *in vivo*, and biodistribution investigation indicated that targeted AuNPs labeled T-cells were mainly migrated to lung and spleen, and these AuNPs were secreted out of body system after two weeks. In a comparison study of CT imaging to fluorescence imaging, T cells dual-labeled with AuNPs and reporter gene overexpressing green fluorescent protein (GFP) demonstrated similar trends of T cells distribution and movement *in vivo.* The incorporated AuNPs also acted as anti-tumor agents in this system, in that targeted T-cells with AuNPs-labeling significantly inhibited tumor progression compared to nontargeted or non-treated controls or AuNPs alone[64].

Other types of labeling

A bimodal-labeled nanoparticle system provides more accurate and quantitative information for cellular tracking compared to single-labeled controls. A dual mode imaging system composed of gadolinium as MRI and aza-BODIPY as NIR was reported to label DCs for *in vivo* tracking[65]. A nanoparticle delivery system can be used to incorporate MRI and NIR agents to enhance the labeling efficiency at low concentrations and to reduce cytotoxicity in DCs. Pittet *et al*. reported the construction of magnetofluorescent-nanoparticle for T-cell labeling. The nanoparticles were composed of MRI contrast agent iron oxide, NIR fluorochromes such as VT680, AF680 or Cy5.5, and HIV-Tat peptide or protamine as clinically viable transfecting agent for effective cellular uptake and displayed membrane-translocating properties[66]. The FePt nanoparticles are reported to play dual roles as contrast agents for both MRI and CT scan[67]. Radionuclide (*e.g.* iodine) labeled AuNPs were quantitatively tracked in dendritic cells by positron emission tomography and Cerenkov luminescence[68,69].

Conclusion

Combining targeted nanoparticles with other conventional approaches such as radiation and surgery is a promising strategy to enhance the immune-based therapeutic or preventing intervention of cancer. Targeting nanoparticle is emerging as a promising tool for immune cell-mediated cancer therapy. The targeting function of nanoparticles can be achieved by tagging a targeting ligand such as receptor-related molecules. Various targeting cells in the immune system have been identified for nanoparticle systems to directly stimulate immune response or suppress the tumor-related microenvironment. The immune responses of targeted nanoparticles for inhibiting tumor growth are well studied both *in vitro* and *in vivo*, but pathways of their cellular uptake, factors influenced the uptake and antigen release, and degradation and secretion pathways of nanoparticles are still in need of further investigation.

Non-invasive imaging by targeted nanoparticles is a potential strategy for understanding these pathways/mechanisms involved in the development of immune therapies. Nanoparticle can be used as carrier systems for fluorescent/bioluminescent probes and contrast agents for MRI and CT, and/or as imaging agents themselves, for imaging immune cells during cancer treatment. However, real-time *in situ* tracking of immune system is still in infancy and much more intensive studies, preclinical and clinical trials are necessary before translating targeted nanoparticles into commercial markets. Outside of cancer treatment, nanoparticles may be used for targeted immunotherapy and imaging of other diseases as well and further studies should be dedicated to exploring this field.

Conflict of interest

The authors declare no potential conflict of interest with respect to the research, authorship, and/or publication of their article.

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