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# Recent progress in nanomaterials for nucleic acid delivery in cancer immunotherapy

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The combination of gene therapy and immunotherapy has the potential to systemically promote anti-tumor effects while reducing adverse reactions. Small interfering RNA (siRNA) has generated great interest in biology, engineering and medicine, especially for cancer treatment due to its ability to knock down genes of interest. Nanomaterials play significant roles in the design of delivery systems of siRNA, and nanomaterial-mediated siRNA delivery in cancer immunotherapy is one of the most important directions for future clinical cancer treatment. Here, we review the recent advances in nanomaterial mediated targeted delivery of siRNA to dendritic cells (DCs), tumor-associated macrophages (TAMs), immune checkpoint inhibitors, B lymphocytes, natural killer cells (NKs), and immunosuppressive cytokines. Fundamental challenges in nucleic acid delivery enabled by bio-barriers, its promising solution strategies and future directions are also reviewed.

## 1. Introduction

Cancer immunotherapy is a major type of cancer treatment that has attracted huge interest over the past few years.[1] Immunotherapy induces long-lasting and systemic anti-tumor immunity, which is especially beneficial for the treatment of recurrent and metastatic tumors.[2,3] However, some patients are less sensitive to immunotherapy, in part because cancer cells do not express sufficient neoantigens.[4] Others may experience severe systemic inflammation and autoimmune side effects.[5,6] One of the main reasons is the on-target/off-tumor toxicity caused by target antigens expressed on normal cells.[7]

Some recent studies have confirmed that a combination of gene therapy and immunotherapy has the potential to promote anti-tumor effects while reducing adverse reactions.[8–10] One of the most successful examples is the T cells expressing CD19 chimeric antigen receptors for sustained remission in lymphocytic leukemia.[9,10] siRNA is a double-stranded RNA with a length of 19–21 nucleotides and has been extensively tested as a potent therapy for cancer in animal models.[11] However, systemic application of siRNA is severely hampered by the complex *in vivo* microenvironment, which causes degradation of the therapeutic molecule, poor penetration in tissue, and low therapeutic outcomes.[12,13] Furthermore, the side effects induced by a cross-reaction between a nucleic acid drug and somatic cells present another major challenge for successful nucleic acid delivery. Therefore, the vector is very crucial to the delivery of siRNA. In the past few decades, siRNA delivery systems have been widely studied as new therapeutic modalities to treat many different types of cancers.[14–17] Viral vector based nucleic acid delivery strategies have demonstrated some success in cancer gene therapy.[18,19] However, the concern of potential insertional mutagenesis, immune stimulation, and other undesired severe side effects hindered their further application. As an alternative strategy, non-viral vectors are used which have many advantages over viral vectors, such as low toxicity, non-immunogenicity, and ease of synthesis.[20–22] Nano-sized non-viral carriers including liposomes, polyethyleneimine (PEI), polypeptides, chitosan, inorganic nanoparticles, *etc.* have been developed as potential excellent nucleic acid delivery vehicles.[23–27] One good example is the nanoparticle-based gene delivery system, CALLA-01, which has been developed for the first phase-1 clinical trial in humans for cancer treatment.[28]

Herein, we highlight some of the achievements and challenges as well as future prospects of non-viral vectors for nucleic acid delivery in cancer immunotherapy and future trends of gene-immunotherapy therapy.

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## 2. Targeted delivery of siRNA to dendritic cells (DCs)

A mature DC is an essential component of immune response, including the following aspects: (a) it is the only proven

antigen-presenting cell that activates naive T lymphocytes; (b) it activates specific T lymphocyte responses against antigens; (c) it is the initiator of the cellular immune response; and (d) it is a bridge connecting innate and acquired immune response.[29] DC-based tumor vaccines are recognized as one of the most potent tumor immunotherapies.[30] Several clinical trials of DC-based vaccine therapy are currently underway.[30] Signal transducer and activator of transcription-3 (STAT3) as an immunosuppressive factor represents a major limitation of DC-based cancer immunotherapy.[31] Activation of STAT3 in DCs inhibits the expression of many immunostimulatory molecules resulting in an immune suppressive microenvironment.[32]

STAT3 inhibits CpG-activated immunostimulation,[33] suggesting a promising therapy combination of CpG with STAT3 siRNA. Yu *et al.* explored a strategy of linking STAT3 siRNA to a CpG oligonucleotide agonist of toll-like receptor 9 (TLR9).[34] The binding of a CpG-siRNA conjugate to the toll-like receptor 9 (TLR9) in DCs led to STAT3 silencing for enhanced antitumor immune response. However, the uptake and gene silencing efficiency of CpG-siRNA were dependent on TLR9 expression. Lim *et al.* synthesized multifunctional hybrid nanoconjugates (HNCs) based on polymer nanoparticles containing quantum dots (QDs) conjugated with CpG oligonucleotides (as a ligand for TLR9) and STAT3 siRNAs (Fig. 1).[35] Hydrophilic CpG ODNs and siRNA molecules were conjugated to QDs so that they were efficiently encapsulated into hydrophobic poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles. Between the QDs and the oligonucleotides, a cleavable disulfide linker (S–S) was introduced to allow for stimuli-responsive cleavage and release of CpG ODNs and STAT3 siRNAs into target cells. Notably, treatment of tumor-tolerant DCs with these NPs successfully blocked STAT3 activation in DCs and promoted CpG ODN based immunostimulation, resulting in synergistically activated antitumor effects evidenced by the increased secretion of TNF-α, IL-6, and IL-12 and significantly suppressed tumor growth *in vivo*.

This system represents potentially useful siRNA delivery nanoplatforms for enhanced DC-based antitumor immunotherapy. It also helps to assess the systemic biodistribution and subcellular localization of nucleic acid-loaded nanoparticles, which helps to gain insight into intracellular trafficking of nanomedicine. A better understanding of the fate of intracellular nanoparticles and the interaction between the parts of a hybrid particle can accelerate nucleic acid delivery-based cancer immunotherapy for clinical use.

Hideyoshi Harashima *et al.* reported a novel cationic lipid, YSK12-C4, for efficient delivery of siRNA in DCs (YSK12-

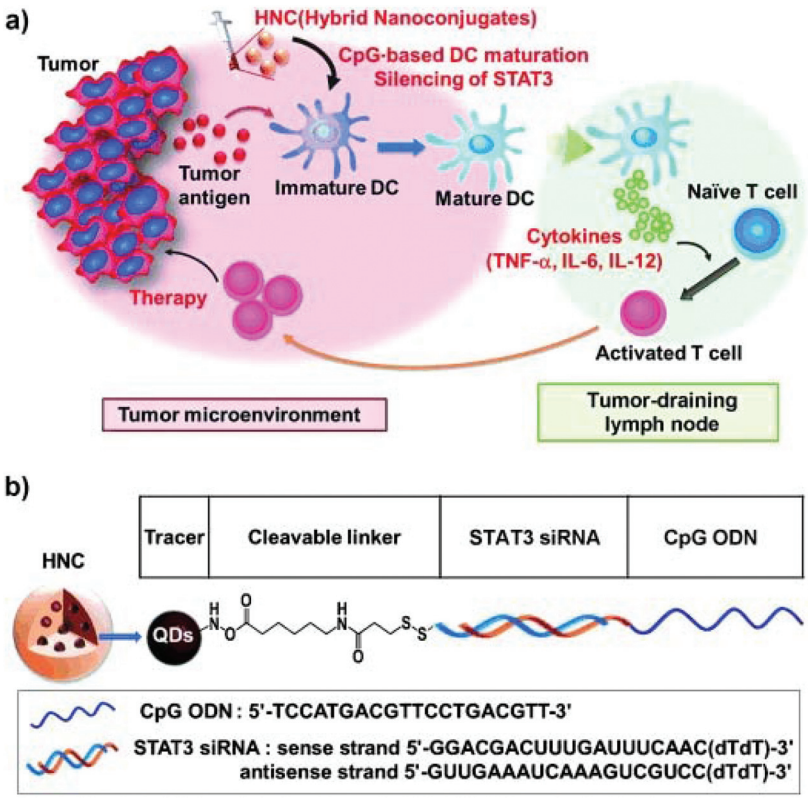


**Fig. 1** (a) Schematic illustration of HNC-based delivery of immunomodulating oligonucleotides to DCs within a tumor microenvironment for the silencing of immunosuppressive genes (STAT3 siRNA) and the activation of TLRs (CpG ODNs), leading to therapeutic antitumor immune responses. (b) Scheme of the composition of HNCs based on polymer nanoparticles containing QDs (as imaging tracers) conjugated to CpG ODNs and STAT3 siRNAs using a cleavable disulfide linker. Copyright 2012 Wiley-VCH Verlag GmbH & Co.

 

MEND).[36] YSK12-MEND revealed higher potency of endosome disruption in comparison with Lipofectamine™ RNAiMAX (RNAiMAX) and R8/GALA-MEND$_{SUV}$.[37] It showed significant gene silencing efficiency of suppressor of cytokine signaling 1 (SOCS1) in mouse DCs, which was superior to RNAiMAX and R8/GALA-MEND$_{SUV}$.[37] Notably, its gene silencing efficiency is comparable to that of lentiviral vectors.[38] All these features resulted in increased production of TNF-α and IL-6, which inhibited tumor growth when it was applied to DC-based immunotherapy in lymphoma-bearing mice. Nevertheless, the cellular uptake of siRNA in YSK12-MEND-treated bone-marrow derived DCs (BMDCs) was not as high as expected. The gene silencing efficiency and immunotherapeutic efficacy can presumably be further enhanced at the improvement of the cellular uptake efficiency of this delivery system.

## 3. Targeted delivery of siRNA to tumor-associated macrophages (TAMs)

TAMs are a class of immune cells that are abundantly present in the microenvironment of solid tumors. They have been proven to be important components of the tumor microenvironment and play an active role in promoting tumor progression.[39–43] TAMs affect many aspects of tumor cell pathophysiology, including tumor cell proliferation, angiogenesis, invasion, metastasis, immunosuppression, and drug resistance.[42,43] Naoki Itano *et al.* outlined the mechanisms responsible for TAM recruitment and highlighted the role of TAMs in the regulation of tumor progression in more detail.[44] Several clinical studies indicate that in many tumor types, high infiltration levels of TAMs, especially M2-like TAMs, are associated with poor prognosis,[45] featuring highly expressed colony stimulating factor-1 receptor (CSF-1R), vascular endothelial growth factor A (VEGF-A), *etc.*[46,47] Therefore, targeted delivery of nucleic acids to M2-like TAMs with optimal non-viral vectors to suppress such receptor expression is a promising strategy for cancer immunotherapy.

It is challenging to deliver nucleic acids specifically to the tumor-promoting M2-like TAMs. Zhang *et al.* developed a dual-targeting nanoparticle delivering siRNA to M2-like TAMs.[47] In this system, the apolipoprotein A1 mimetic (α-peptide) acts as a ligand for SR-1B (a scavenger receptor B type 1), which is linked to an M2 macrophage binding peptide (M2pep) to deliver NP-encapsulated anti-CSF-1R siRNA (siCD115) (Fig. 2). This molecular targeting strategy increased the expression of CD8$^+$ T cells at a factor of 2.9 in the tumor microenvironment (TME). Furthermore, M2NPs loaded with siRNA down-regulated the expression of depletion markers (PD-1 and Tim-3) on infiltrating CD8$^+$ T cells. M2NPs also stimulated their secretion of IFN-γ (6.2-fold). On day 19 after tumor inoculation, it was observed that the M2NP-siCD115 group significantly reduced tumor growth by 87% compared to the phosphate buffered saline (PBS) group. All of these pieces of evidence suggested that targeting the M-CSF receptor with siCD115 in TAMs led to the restoration of T cell anticancer immunity. A nucleic acid delivery system targeting M2-like TAMs was developed, resulting in the growth inhibition of B16 melanoma *in vivo*. Zhang *et al.* developed a galactosylated acid-responsive cationic dextran nano-complex containing a CpG oligonucleotide, and anti-IL-10 and anti-IL-10 receptor oligonucleotides.[48] Their studies indicated that this nucleic acid delivery system enhanced antitumor efficacy without affecting systemic immunity.

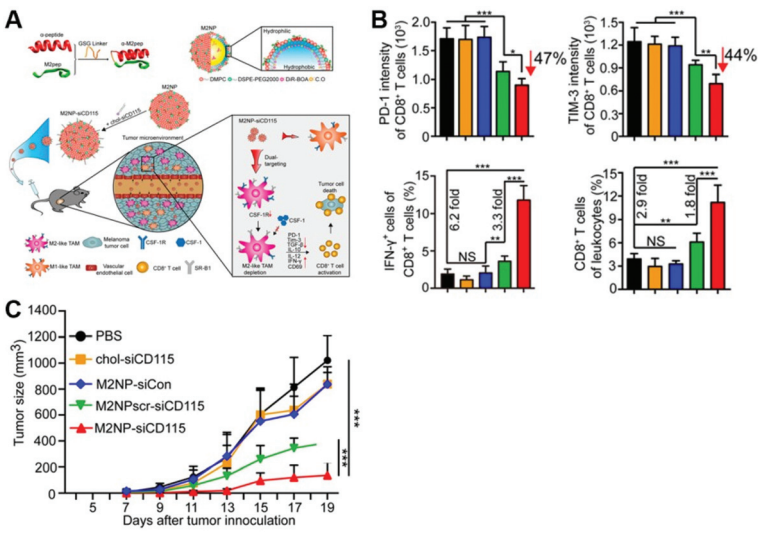


**Fig. 2** (A) Design of the M2NP for M2-like TAM-specific molecular-targeted immunotherapy. (B) PD-1 and Tim-3 expression on CD8+ T cells, IFN-γ secretion of tumor-infiltrating CD8$^+$ T cells, and proportion of CD8+ T cells among the total tumor-infiltrating leukocytes in mice after the indicated treatment, $n$ = 6 mice per group. (C) Tumor growth curves of B16 tumors in C57BL/6 mice treated with PBS, chol-siCD115, M2NP-siCon, M2NPscr-siCD115, or M2NP-siCD115; $n$ = 6 mice per group. Copyright © 2017 American Chemical Society.

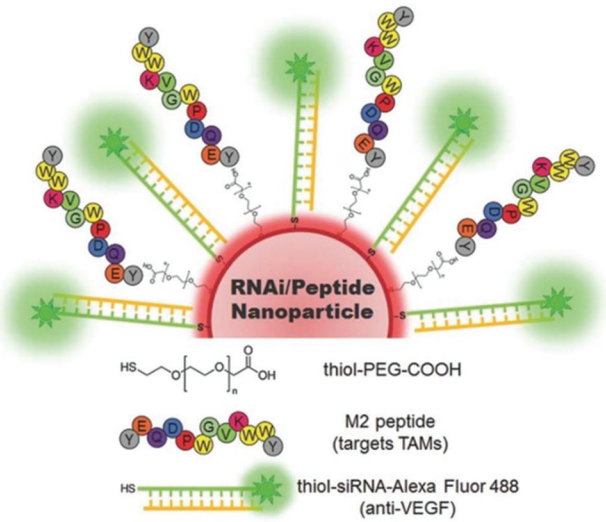


Fig. 3 Gold nanoparticles (AuNPs, ≈15 nm) functionalized with thiolated-PEG-COOH conjugated to the TAM-targeting peptide (M2pep) and thiolated anti-VEGF siRNA labeled with Alexa Fluor 488. Copyright 2015 Wiley-VCH Verlag GmbH & Co.

M2-like TAMs can inhibit anti-tumor immune responses and stimulate angiogenesis, leading to malignant progression of the tumor.[49,50] Tian *et al.* reported gold nanoparticles functionalized with thiolated PEG-COOH, to which TAM-targeting peptide (M2pep) and thiolated anti-VEGF siRNA were conjugated (Fig. 3).[51] This nanoparticle-based strategy can specifically silence VEGF in TAMs and lung cancer cells. This hybrid approach demonstrates that efficient dual knockdown in cancer cells and in TAMs in the TME will result in viable, highly effective anti-cancer immunotherapy.

## 4. Delivery of siRNA to regulate T cell function

Currently, one of the most promising types of cancer immunotherapy is based on immune checkpoint inhibitors. Checkpoint blockade-based immunotherapy is a promising clinical approach to combat metastatic tumors by activating tumor-specific T cells.[52,53] Several therapeutics have been approved by the U.S. Food and Drug Administration (FDA), such as the anti-cytotoxic T-lymphocyte-associated protein 4 (anti-CTLA-4) monoclonal antibody ipilimumab,[54] the anti-PD-1 antibody pembrolizumab,[55] and the anti-PD-L1 antibody atezolizumab.[56] Despite the significant success of checkpoint inhibitors in cancer treatment, individual patient response varied significantly depending on cancer types and the immune checkpoint expression. The severe systemic side effect is also a big concern. Thus, the non-viral vector based direct regulation of T cells by targeting immune checkpoint pathways *in vivo* is a promising choice to tackle such challenges. Some recent progress has been made in siRNA delivery to T cells by using nanoparticles,[57,58] but many more obstacles are still present between the state of the art and bed-side translational outcomes of this technology.[59]

### 4.1 CTLA-4 siRNA delivery *via* non-viral vectors

Wang *et al.* prepared siCTLA-4 encapsulated nanoparticles ($NP_{siCTLA-4}$) with PEG-PLA (poly(ethylene glycol)-*block*-poly(D,L-lactide)) and cationic lipid BHEM-Chol (*N*-bis(2-hydroxyethyl)-*N*-methyl-*N*-(2-cholesteryloxycarbonyl aminoethyl) ammonium bromide) by the double emulsion method (Fig. 4).[60] $NP_{siCTLA-4}$ were used to deliver siRNA specific to CTLA-4 into T cells, which enhanced the activation and proliferation of T cells. In this study, increased T cell-mediated anti-tumor immune

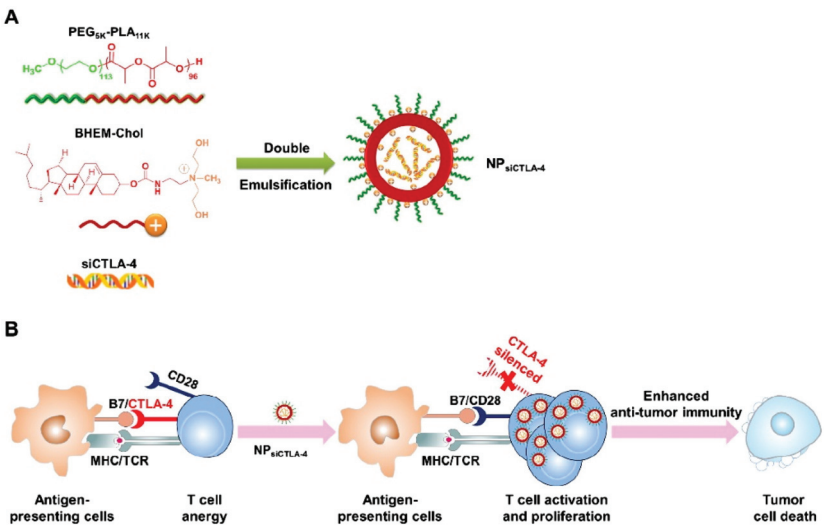


Fig. 4 (A) Preparation of siCTLA-4-encapsulated nanoparticles (NPsiCTLA-4) with poly(ethylene glycol)-*block*-poly (D,L-lactide) and a cationic lipid BHEM-Chol by double emulsification. (B) Enhancing T cell-mediated immune responses by blocking CTLA-4 using NPsiCTLA-4. CTLA-4 plays a strong inhibitory role in T cell activation and proliferation, which significantly curbs T cell-mediated tumor rejection. NPsiCTLA-4-mediated CTLA-4 knockdown enhanced the activation and proliferation of T cells, which inhibited the overall growth of tumors. Copyright 2016 Elsevier.

response was induced in melanoma-bearing mice receiving $NP_{siCTLA-4}$ compared with mice receiving control nanoparticles. In addition, $NP_{siCTLA-4}$ effectively inhibited tumor growth and prolonged the survival of melanoma-bearing mice.

### 4.2 PD-L1 siRNA delivery *via* non-viral vectors

Li *et al.* integrated a PDPA-OEI-C14-PPa hybrid micelle (POP micelle), a photosensitizer (PS) and siRNA.[61] POP is an acid-activatable cationic micelle for siRNA complexation and delivery. The synthetic POP-PD-L1 micelle complex showed a synergistic therapeutic effect in inhibiting tumor growth in B16-F10 melanoma-bearing mice. In addition to combining PDT, nanoparticle-based nucleic acid delivery for immunotherapy combined with chemotherapy, radiation therapy, photothermal therapy, or other methods, may bring huge opportunities for cancer treatment. Combination therapy has the potential to enhance the efficacy of immunotherapy, making tumor cells more vulnerable to attack by the immune system.

## 5. Targeted delivery of siRNA to B lymphocytes (B cells)

B cells can regulate immunity, including antibody production, cytokine secretion, T-cell activation, and memory cell generation. Despite their role in regulating immune responses, B cell dysfunction may lead to autoimmune disorders and malignancies. Therefore, B cells represent an attractive target for nucleic acid delivery for immune disease prevention and treatment. However, the lack of strategies for delivering nucleic acids to the B cells hinders the development of RNAi-based therapeutics. Peer *et al.* designed an efficient and non-immunogenic system for the delivery of nucleic acids into B cells *in vivo*.[62] They used αCD38 antibody-LNPs encapsulating cyclin D1 (CycD1) siRNA to inhibit CycD1 expression in a human mantle cell lymphoma xenograft mouse model. Their results showed downregulation of CycD1, inhibition of tumor growth and prolonged survival, indicating that specific delivery to B cells can be achieved by encapsulating siRNA into LNPs coated with a targeting antibody. Delivery of the siRNA cargo encapsulated in LNPs opens a new avenue for treating B-cell malignancies with siRNA.

## 6. Targeted delivery of siRNA to natural killer cells (NKs)

NKs, cytotoxic lymphocytes critical to innate and adaptive immunity, play a vital role in tumor immune clearance and immunosurveillance to induce tumor cell death. Unlike T cells or B cells, NKs do not require specific antigen stimulation to trigger the killing of target cells. However, NK cell-based immunotherapy has been tempered due to the short post-infusion persistence of NK cells and their ability to migrate to tumor tissues *in vivo*. Though nanoparticles have been widely used for nucleic acid delivery, the use of nanoparticles for genetic reprogramming of NK cells is still in its infancy. Recently, researchers have become increasingly aware of the importance of NKs in immunotherapy. In addition to DCs, Harashima *et al.* also used nanoparticles (YSK12-MEND) to deliver siRNA into NKs.[63] YSK12-MEND encapsulating siGAPDH was more effective in downregulating GAPDH in NK92 cells compared to the Lipofectamine® RNAiMAX reagent, 75% and 19%, respectively. The authors believed that this was likely due to the small size and non-aggregability of nanoparticles, which enhanced their accessibility for NKs in the medium. However, significant toxicity by YSK12-MEND was observed in NKs at the siGAPDH dose required to achieve sufficient gene silencing. The authors suspected that this might be related to the cationic head YSK12-C4, suggesting the necessity of reducing the content of YSK12-C4 in MEND. They introduced a core complex formed by electrostatic interactions of siRNA with a polycation (protamine) (siRNA core) to the YSK12-MEND to reduce the total amount of the cationic lipid.[64] It decreased the cytotoxicity in NKs while maintaining gene silencing efficiency. The use of YSK12-MEND/core is expected to represent a highly promising approach for the delivery of nucleic acids to NKs *in vivo* and in the clinic.

## 7. Delivery of siRNA targeting the immunosuppressive cytokine

Immunotherapy has become an attractive strategy and an important part of successful anti-tumor therapy.[65] However, cancer vaccines failed to meet initial expectations when used against aggressive and advanced malignancies.[66] To develop an effective method for treating advanced tumors, Huang's group used liposome-protamine-hyaluronic acid (LPH) NPs to deliver siRNA against TGF-β and lipid-calcium phosphate (LCP) NPs to deliver tumor antigen and CpG oligonucleotide.[67] LPH NPs were prepared by a step-by-step self-assembly process based on their previously established protocol.[68] It has been optimized for delivering siRNA to the tumor site specifically and efficiently.[68] The results indicated that LPH NPs carrying siRNA against TGF-β led to knockdown of TGF-β and reversal of the immunosuppressive microenvironment in a melanoma mouse model (Fig. 5). Delivery of cytokine-targeted siRNA (TGF-β siRNA) is expected to promote the efficacy of therapeutic cancer vaccines *via* reducing immunosuppressive cytokine secretion and generating an antitumor response.

## 8. Multifunctional nanoparticles for siRNA delivery combination therapy

Single function nanoparticle-mediated nucleic acid delivery typically only prevents localized cancer, while multifunctional nanoparticles have the ability to resist metastatic cancer. Over the past few decades, various nanomaterials with strong near-

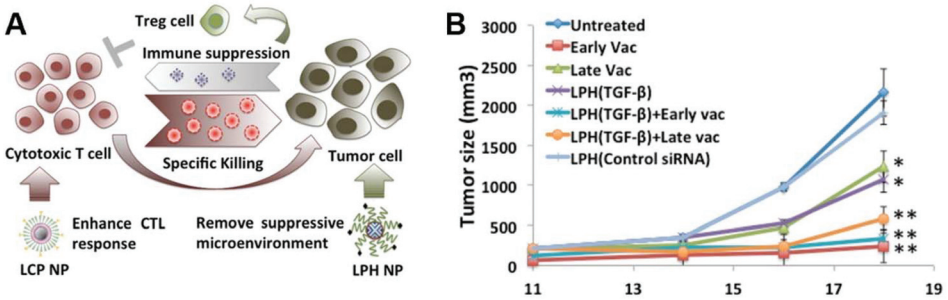


**Fig. 5** (A) Schematic illustration of nanoparticle-delivered transforming growth factor-β siRNA enhances vaccination against advanced melanoma by modifying the tumor microenvironment. (B) C57BL/6 mice were inoculated with $2 \times 10^5$ B16F10 cells SC on day 0. LCP vaccine was given on day 4 (early vac) or day 13 (late vac). LPH NPs containing siRNA (0.6 mg $kg^{-1}$) against TGF-β were injected intravenously on days 13, 15, and 17. Tumor growth was measured every 2 to 3 days for 18 days. $n = 5$, $^*P < 0.05$, $^{**}P < 0.001$. Statistical analyses were performed by comparing with the untreated group unless specified with markings. Copyright © 2014 American Chemical Society.

infrared (NIR) absorbance have shown great promise in photodynamic or photothermal treatment of cancer, achieving encouraging therapeutic efficacies in many *in vivo* animal studies. Laser-induced tumor cell death can release tumor antigens into the surrounding environment to elicit specific antitumor immunity. Nanoparticles for nucleic acid delivery provide a hybrid platform that performs multiple functions.[69] Exploring multifunctional nanoparticles in nucleic acid delivery has the potential to make significant advances in the treatment of cancer immunotherapy, particularly in metastatic cancer. Here we highlight the important progress of multifunctional nanoparticle mediated siRNA delivery in combination with photodynamic therapy (PDT), photothermal therapy (PTT) or chemotherapy.

### 8.1 siRNA delivery combined with photodynamic therapy (PDT)

Depending on the type of cancer, there could be up to 100 mutations leading to amino acid changes in any tumor tissue.[70] It is unlikely to completely treat tumors with a single therapeutic agent. Nanoparticle-based gene therapy in combination with chemotherapy, radiation therapy, photodynamic therapy, immunotherapy or other methods have the potential to be one of the most important methods in future clinical implementation.

Tumor cell immunosuppression of host T cell antitumor activity severely impairs PDT-mediated cancer immunotherapy by the PD-L1 and PD-1 immune checkpoint pathway. To overcome these obstacles, Cai *et al.*[71] reported a micellar nanocomplex co-loaded with PD-L1-targeting siRNA (siPD-L1) and a photosensitizer (MTPP) in the core. The structure of pH-responsive, PEG-coated nanocomplexes made of PEG-CDM-PDEA (poly(2-(diethylamino) ethyl methacrylate)) and PEI-PDEA (denoted as PCPP) can be disrupted in the acidic tumor microenvironment, which leads to significant size reduction and increase of positive charge (Fig. 6). These transitions promote the penetration and uptake of siPD-L1 to tumor cells. The PEI-conjugated PDEA copolymer (PEI-PDEA) has strong siRNA binding affinity and a sponge effect in endo/lysosomes. Results from *in vitro* and *in vivo* experiments together revealed that the nanocomplex synergistically activated the PDT-induced immune response and silenced the immune resistance mediated by PD-1/PD-L1 interactions. This study provides an alternative strategy for developing effective nucleic acid delivery to improve antitumor immunotherapy.

### 8.2 siRNA delivery combined with photothermal therapy (PTT)

Oligodeoxynucleotides containing the cytosine–guanine (CpG) motif can be effective as monotherapy and as vaccine adjuvants for cancer immunotherapy.[72,73] However, *in vivo* instability, adverse pharmacokinetic reactions, *etc.* hinder the clinical application of CpG oligodeoxynucleotides. PTT uses the heat generated by the light energy absorbed by the light absorbers accumulated in the tumor to disrupt tumor cells.[74,75] However, photothermal therapy also has its shortcomings. In currently used forms, nanoparticle-mediated photothermal ablation is less effective in controlling metastatic cancer.

Lu *et al.* designed a near-infrared light-responsive transformative nano-CpG platform, hollow CuS nanoparticles-CpG (HCuSNPs-CpG), for cancer photothermal immunotherapy.[76] HCuSNPs were coated with chitosan to conjugate with CpG oligodeoxynucleotides that specifically activated toll-like receptor 9 (TLR9) signaling in plasmacytoid DCs. Under laser irradiation, these hollow nanoparticles were broken down into small CuS nanocrystals that tend to reassemble and transform into chitosan–CpG nanocomplexes, which promoted CpG uptake by plasmacytoid DCs (Fig. 7). HCuSNP-CpG-mediated photothermal immunotherapy led to a comprehensive anticancer effect against both local and metastatic tumors.

### 8.3 siRNA delivery combined with chemotherapy

Systemic chemotherapy remains the primary treatment for advanced cancer with limited efficacy. Traditional che-

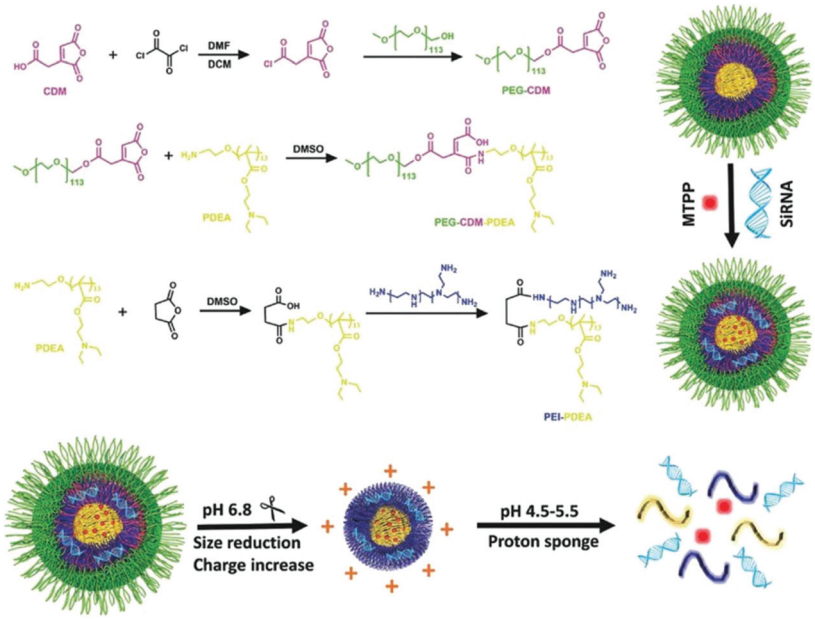


**Fig. 6** Synthesis routes of a pH-responsive dissociable micelleplex of PCPP, and the mechanism of drug/siRNA release from polymeric micelleplexes under acidic pH. Copyright 2018 Wiley-VCH Verlag GmbH & Co.

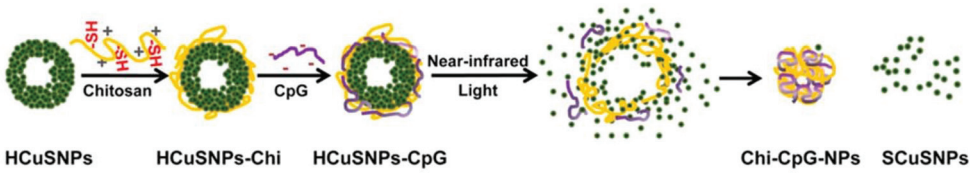


**Fig. 7** Scheme of the assembly of HCuSNP–CpG conjugates, near-infrared light-triggered disintegration of HCuSNPs, and system reassembly. "HCuSNPs-Chi" represents chitosan-coated HCuSNPs. "Chi-CpG-NPs" represent chitosan-CpG nanocomplexes. "SCuSNPs" represent small CuS nanoparticles. Copyright © 2014 American Chemical Society.

motherapeutic agents have the disadvantages of strong toxicity, poor targeting, and being prone to drug resistance. To increase the efficacy of chemotherapeutic approaches without causing these disadvantages, a combination of chemotherapy and immunotherapy has been clinically evaluated. Great numbers of clinical trials have demonstrated that combining immunotherapy with chemotherapy has a synergistic effect and improved efficacy, without novel toxicities.[77–81]

Jon *et al.* established a vector that delivered both immunostimulatory and cytotoxic chemotherapeutic agents.[82] In this system, single-strand DNA–A9 PSMA (prostate-specific membrane antigen) RNA aptamer hybrids were conjugated to dendrimeric nanostructures. A plasmid bearing unmethylated CpG was used as both an immunostimulatory agent and a carrier of the chemical drug, Dox. PSMA can specifically target PSMA-overexpressing prostate cancers. The results indicate that Dox@Apt·dONT-DEN has stronger resistance to prostate cancer cells and xenograft tumor models than the same dose of free-Dox or an aptamer-free dendrimer conjugate (Dox@dONT-DEN).

Lim *et al.* developed a system utilizing a hyaluronic acid–paclitaxel (HA/PTX) complex and PLGA loaded respectively with TLR-based cytosine–phosphate–guanosine oligodeoxynucleotides (CpG ODNs) and IL-10 siRNA.[83] It is hypothesized that the initial injection of HA/PTX causes tumor cell death and tumor-associated antigen generation. The released tumor-associated antigen was thought to be taken up by tumor-recruited bone marrow-derived dendritic cells (BMDCs). CpG ODNs were used to enhance immune response. However, CpG ODNs also induced the secretion of immunosuppressive cytokine IL-10. IL-10 siRNA was used to inhibit IL-10 secretion from BMDCs to further enhance the immune response. As a result, the sequential treatment not only effectively inhibited tumor growth but also improved the animal survival rate. Their results suggest that the combination of a chemotherapeutic agent and immunomodulatory nanomaterials represents a promising strategy for efficient cancer treatment.

## 9. Challenges

Although significant advances have been reported in nucleic acid delivery for cancer immunotherapy, there are still many fundamental challenges that restrict the widespread application of non-viral vectors. The intrinsic properties of nucleic acids prevent their direct applications *in vivo*. The internalization efficiency of nucleic acids is very low because of their negative charge. The internalized nucleic acids tend to be engulfed by the reticuloendothelial system. The naked nucleic acids are susceptible to rapid degradation by plasma and tissue nucleases. Endosomal retention prevents it from reaching the cytosol for intended therapeutic functions. Like other drugs, delivery of nucleic acids needs to overcome various other tough physiological barriers, including the blood–brain barrier and blood tumor barriers in a hypoxic environment. As mentioned above, progress has been made in developing optimal non-viral vectors for nucleic acid delivery.[84] However, many efforts have focused on solving one or two problems at a time, while others have emerged. Integrating various characteristics is of vital importance so that the next-generation nucleic acid delivery systems will be empowered to overcome a variety of biological barriers.

A 'perfect' non-viral nucleic acid delivery platform should simultaneously achieve a compact size, good cell penetration, *in vivo* stability, high payload, low immunogenicity, low cytotoxicity, selective targeting, efficient endosomal escape, and ease of production. The application of nanoparticle-based nucleic acid therapy in the clinical practice of cancer immunotherapy still has a long way to go.

## 10. Conclusion

Combining gene therapy with immunotherapy through nanoparticles may reduce the incidence of drug resistance and produce a synergistic therapeutic effect. Recently, several strategies have been devised to improve the efficiency of nucleic acid delivery in cancer immunotherapy. Results show that nucleic acid-loaded DC-specific, TAM-specific, T cell-specific, B cell-specific or NK cell-specific nanoparticles have the potential to promote immunotherapy to its full potential. The successful development of nanoparticle-based nucleic acid delivery platforms in the field of immunotherapy will allow the application of vaccines, adjuvants and immunomodulatory drugs that improve clinical outcomes for cancer immunotherapy. However, non-viral vectors can only transmit certain genes to certain cells, and the nucleic acid delivery efficiency of the same nanoparticle is heterogeneous in different tumors and different mouse models, let alone humans. Moreover, another problem is drug resistance caused by the delivery of nucleic acids with nanoparticles. Thus, a novel broad-spectrum nucleic acid delivery non-viral vector without drug resistance needs to be developed. The Cancer Genome Atlas (TCGA) shows that many cancers are caused by co-mutations of multiple genes. The effectiveness and persistence of cancer treatment may be further enhanced by the simultaneous delivery of different nucleic acids by the nanoparticles. Recently, long non-coding RNA (lncRNA) and circRNA have been proved to play an important role in tumor progression.[85,86] Studying non-viral vectors for nucleic acid delivery to inhibit the expression of lncRNA and circRNA may play a key role in conquering cancer. To facilitate the clinical application of nucleic acid delivery nanoparticles in cancer immunotherapy, collaboration among materials scientists, basic medical scientists, clinicians, and molecular biologists is needed.

## Conflicts of interest

There are no conflicts to declare.

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