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Discussion

Nanomaterials for cancer immunotherapy, what is the next?

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ABSTRACT

Immunotherapy, although effective, may have a low response rate and off-target toxicity when used to boost the immune system in cancer treatment. However, the use of nanomaterials has revolutionized cancer treatment by enabling precise drug delivery, improving the efficacy of cancer vaccines, and manipulating the immune activity of macrophages and T cells.

Introduction

Immunotherapy has established itself in the clinic as the fourth pillar of cancer treatment, along with surgery, chemotherapy, and radiotherapy. Cancer immunotherapy aims to harness the host immune system to detect and destroy cancerous cells, leading to improved systemic immune surveillance and eradication of metastatic tumors. Immunotherapy can also maintain a long-term immune memory that protects against tumor recurrence. Despite the great progress achieved in the clinic, cancer immunotherapy still faces challenges, such as low response rates, limited efficacy, and considerable toxicity. Nanomaterial-based nanomedicine, which involves formulating immunostimulant agents in nanocarriers, presents a promising solution to these challenges. Lipid, polymer, nanohydrogel, and bioinspired materials are some examples of nanomaterials that have been widely applied to improve cancer immunotherapy and have shown promising results in preclinical studies and clinical trials. In this comment, we describe three major challenges in immune modulation and discuss possible solutions in the form of engineering nanomaterials.

Delivery of immunomodulatory drugs

The first challenge in cancer treatment is to safely and effectively modulate the immune system using immunomodulatory drugs such as interleukin-2 (IL-2), interferon-α (IFN-α), IL-12, IL-15, anti-CD28 antibody, anti-CD137 antibody, anti-CD47 antibody, stimulator of interferon genes (STING) agonists, and immune checkpoint blockers (anti-PD-L1, anti-PD-1, or anti-CTLA-4 antibody). However, systemic administration of these drugs is accompanied by serious immune-related adverse events as they disrupt the functions of immune cells at non-target sites. Intratumor injection can improve drug concentrations in tumors while reducing toxicity, but rapid diffusion into circulation leads to toxicities similar to those following systematic injection [1,2]. Nanomaterials offer a promising approach to enhancing both the efficacy and safety of immunomodulators in cancer immunotherapy. By engineering nanomaterial surfaces, immunomodulators can be targeted at specific cell types and anatomical locations.

The use of smart nanomaterials in drug delivery systems enables targeted immunodrug delivery and controlled payload release in response to various stimuli such as acidic/hypoxic environments, enzyme levels, glutathione, and metabolic changes (Fig. 1a). For example, using pH-sensitive polymers encapsulating a STING agonist, 2′3′-cGAMP, endosomolytic polymersomes can enhance 2′3′-cGAMP activity in immune cells by two to three orders of magnitude through acid-mediated endosomal escape of payloads, transforming immunosuppressive tumors into immunogenic and tumoricidal environments [3]. When injected intratumorally or intravenously, these polymersomes significantly increase the inhibitory effect on B16-F10 tumor growth and improve the response to immune checkpoint inhibitors, including anti-PD-1 antibody and anti-CTLA-4 antibody. Similarly, lipid nanodiscs incorporating cGAMP molecules enable superior tumor penetration and improved $CD8^+$ T cell priming compared with a high dose of free cGAMP (20-fold) or cGAMP encapsulated in liposomes [4]. A single intravenous injection of these nanodiscs results in substantial rejection of established MC38, 4T1, and TC1 tumors and a potent long-term immune memory against tumor recurrence.

Apart from systemic administration, nanomaterials can also be locally injected or implanted with immunomodulators to improve

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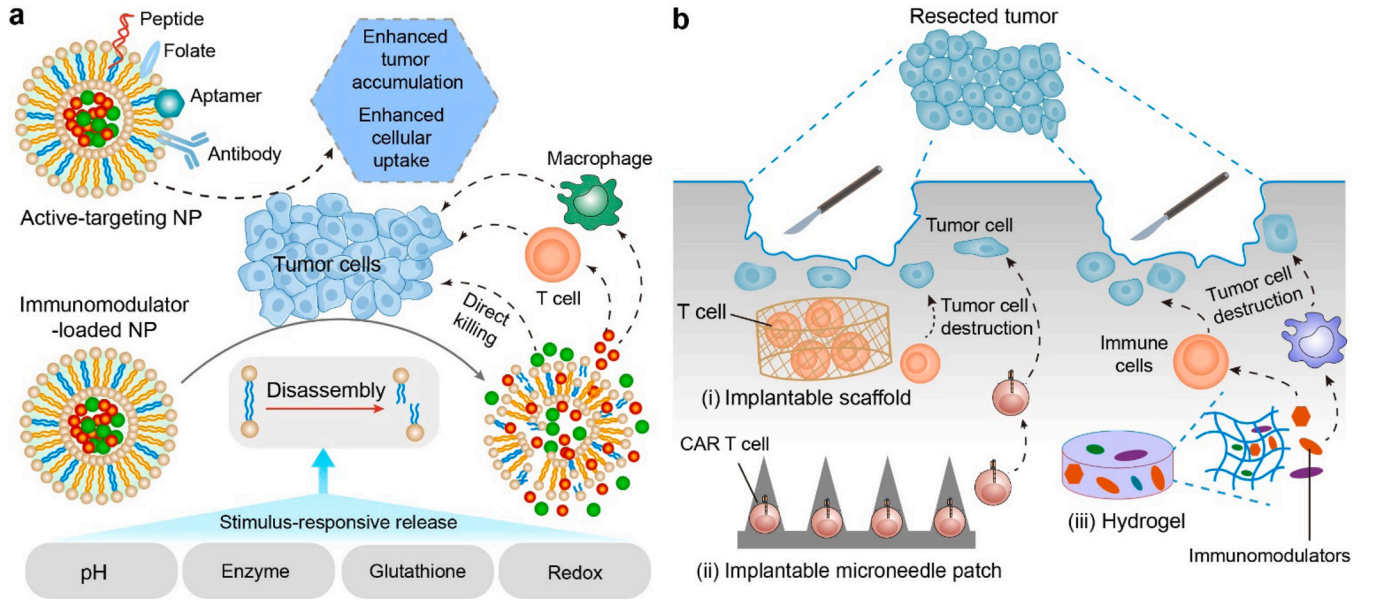

Fig. 1. Nanomaterials improve cancer immunotherapy by facilitating the delivery of immunomodulatory drugs. a, Nanomaterials can be targeted for delivery to tumor cells, with the ability for stimulus-responsive release at tumor sites. **b**, Various types of nanomaterials such as implantable scaffolds (i), microneedle patches (ii) and hydrogels (iii), can be used to locally deliver immunomodulators directly to the surgical cavity, thus enhancing tumor eradication post-surgery.

their antitumor activities by controlled release, especially against tumor recurrence and metastasis (Fig. 1b). One example of this is a bio-responsive hydrogel that is formed by the interaction of thrombin and fibrinogen, incorporated with $CaCO_3$ nanoparticles coated with anti-CD47 antibodies and implanted into the surgery cavity. This hydrogel significantly suppresses local tumor recurrence and metastatic spread through M1-type macrophage-activated innate and adaptive immunity [5]. Another example is the *in situ* implant of platelet-derived microparticles loaded with anti-PD-L1 antibody, which also shows significant reduction in post-surgical tumor recurrence and metastasis in both B16-F10 and 4T1 tumor models by enhancing the delivery of anti-PD-L1 antibody to the surgical bed and target circulating tumor cells in the bloodstream [6].

Boosting the therapeutic efficacy of cancer vaccines

Nanomaterials have shown promise in improving the efficacy of cancer vaccines by addressing the challenge of low response rates and weak immune response in clinical settings. Various types of nanomaterials, such as polymeric nanoparticles, inorganic nanoparticles, liposomes, nanogels, virus-like particles, and self-assembled peptide-based nanoparticles, have been used to improve the retention time of vaccine components (antigen and adjuvant) in lymph nodes. By delivering the antigen and adjuvant concurrently to the same antigen-presenting cell (APC), nanomaterials can promote antigen-presentation by APCs and T cell priming, generating strong antigen-specific cytotoxic T lymphocyte (CTL) responses (Fig. 2a). For instance, nanovesicles that are crosslinked between interbilayers and contain ovalbumin (OVA) as an antigen and monophosphoryl lipid A (MPLA) as an adjuvant, can produce 14 times more SIINFEKL-$CD8^+$ T cells in the peripheral blood than a mixture of soluble OVA and MPLA (Ref. [7]). Moreover, synthetic high-density lipoprotein nanodiscs coupled with Adpgk neoantigen and CpG oligonucleotide as an adjuvant can evoke 47 times more antigen-specific CTLs than a mixture of soluble Adpgk and CpG, thereby eliminating established B16-F10 tumors [8].

Nanomaterials can also function as adjuvants on their own, which simplifies vaccine production. Self-adjuvanted nanomaterials facilitate the delivery of antigens to the cytosol, promoting major histocompatibility complex class I (MHC-I) presentation and boosting cellular immunity (Fig. 2b). Several studies have demonstrated the effectiveness of self-adjuvanted nanomaterials in inhibiting tumor growth and preventing post-operative tumor recurrence and metastasis in various tumor models. For example, self-adjuvanted and pH-sensitive polymeric nanoparticles (PEG-PC7A) loaded with tumor antigens showed potent tumor inhibition in melanoma, colon cancer, and human papillomavirus (HPV)-E6/E7 tumor models *in vivo*, resulting from the increased cytosolic antigen delivery and STING-activating effect of the PC7A segment [9]. A nanovaccine formed by mixing fluorinated polyethyleneimines (PEIs) with OVA can induce dendritic cell (DC) maturation *via* the toll-like receptor (TLR) 4-signalling pathway and promote the release of the loaded antigens into the cytosol of DCs to induce efficient MHC-I presentation, eliciting a high level of antigen-specific CTL responses *in vivo* [10]. These enhanced CTL responses significantly suppress established B16-OVA tumors, and such fluoropolymer-based personalized cancer vaccines, in combination with immune checkpoint blockade, effectively prevent post-operative tumor recurrence and metastasis. On a separate development, a proton-driven nanotransformer cancer vaccine based on amphiphilic polymer (p($DMAEMA_{22}$-$OGEMA_4$)-*b*-p$(MAVE)_{30}$)-peptide conjugates effectively inhibits the growth of B16-F10 and HPV-E6/E7 tumors, as a result of the vaccine-induced robust cellular immunity [11]. In the acidic endosomal environment, the morphology of these nanovaccines changes dramatically from nanospheres to nanosheets, which directly promotes endosomal membrane disruption to release antigens into the cytosol of DCs while simultaneously inducing DC maturation *via* the NLRP3 inflammasome pathway.

Nanomaterials can endow cancer vaccines with antigen self-presentation and immunosuppression reversal functions (Fig. 2c), which presents another promising strategy to improve the therapeutic efficacy of cancer vaccines. A bioengineered nanovaccine, derived from recombinant adenovirus-infected dendritic cells, enables the directional presentation of specific antigenic epitopes by MHC-I molecules and the co-delivery of anti-PD-1 antibodies and B7 co-stimulatory molecules *via* a programmed process [12]. Such nanovaccine can not only present antigenic epitopes directly to $CD8^+$ T cells, triggering strong CTL responses, but also break immune tolerance through the anchored checkpoint inhibitor anti-PD-1 antibody, effectively eliminating established tumors and providing long-term immunoprotection.

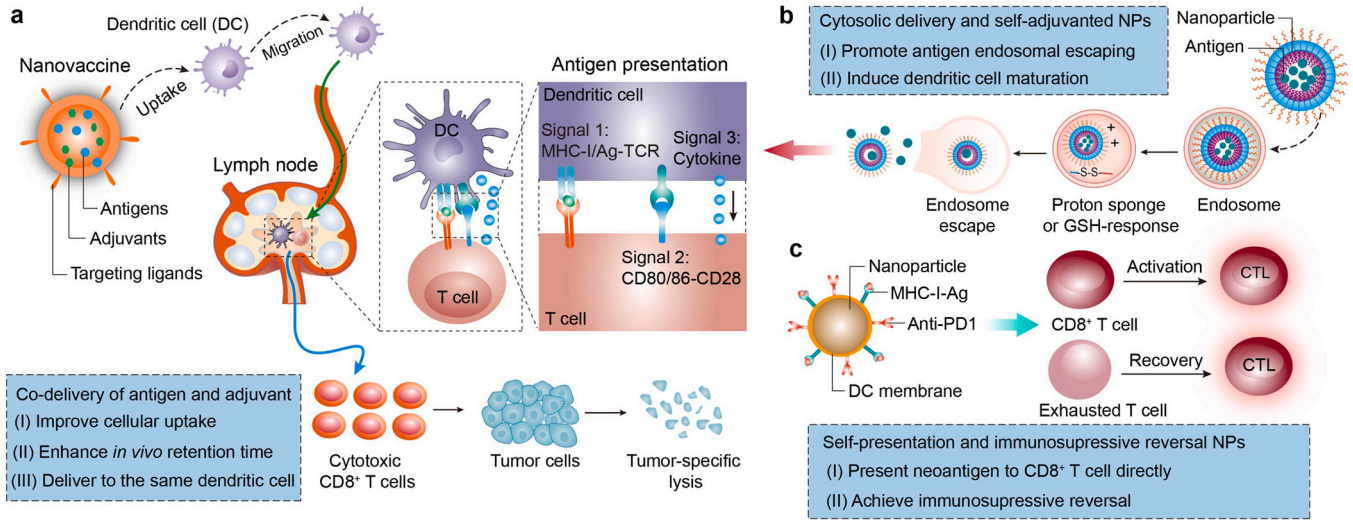

Fig. 2. Nanomaterials improves the efficacy of cancer vaccines through various mechanisms. a, Nanomaterials can efficiently co-deliver antigen and adjuvant to the same dendritic cell, a key player in initiating and regulating immune responses. **b**, Self-adjuvanted and cytosolic-delivery nanomaterials can promote MHC-I antigen presentation through endosomal escaping effect. This allows for the generation of strong cytotoxic $CD8^+$ T cells capable of killing tumor cells. **c**, Nanomaterials can be designed to directly present neoantigens to $CD8^+$ T cells and to overcome immune tolerance. Neoantigens are unique antigens that arise from mutations in tumor cells and are not present in normal cells. By presenting neoantigens to $CD8^+$ T cells, nanomaterials can stimulate a strong cytotoxic T lymphocytes (CTL) response against cancer cells.

Direct modulation of immune cells

Nanomaterials have the potential to improve the efficacy of tumor immunotherapy by interacting directly with macrophages or T cells (*e.g.*, CAR T cells). While macrophages are essential for cancer cell identification and degradation, macrophage-mediated immunotherapy has a low response rate in solid tumors due to the lack of membrane-bound proteins such as signaling lymphocytic activation molecule family member 7 (SLAMF7). To address this issue, bispecific tumor-transforming nanoparticles ($BiTN_{HER}$) have been developed by conjugating polymeric nanoparticles (poly(ethylene glycol)-poly(D, L-lactic-co-glycolic acid): PEG-PLGA NPs) with an anti-human epidermal growth factor receptor 2 (HER2) antibody as a tumor cell targeting ligand and recombinant SLAMF7 protein as a macrophage targeting ligand. This approach enhances macrophage-mediated phagocytosis of tumor cells and thus improves the antitumor effect of cancer immunotherapy in solid tumors [13]. When combined with CD47 blockade, $BiTN_{HER}$ significantly suppresses the growth of tumors with HER2 expression in 4T1 and TUBO tumor models by activating the STING pathway in intratumoral macrophages.

T cell-based cancer immunotherapies, such as adoptive T cell therapy (ACT), chimeric antigen receptor (CAR) T cell therapy, T cell receptor engineered-T-cell (TCR-T) therapy, and tumor infiltrating lymphocyte (TIL) therapy, have shown promise and have been approved by the US Food and Drug Administration for clinical use in certain cancers, including tisagenlecleucel (ACT for B cell leukemias), axicabtagene ciloleucel (ACT for B cell lymphomas), and brexucabtagene autoleucel (CAR T cell therapy for Mantel Cell lymphoma). However, the effectiveness of these therapies is limited by challenges such as T cell exhaustion and death caused by the immunosuppressive microenvironment. To overcome this, researchers have developed T cell-targeting fusogenic liposomes with 2, 2, 6, 6-tetramethylpiperidine (TEMP) groups that neutralize reactive oxygen species in the tumor microenvironment, protecting T cells from oxidation-induced loss of their tumor-killing activity [14]. Moreover, the paramagnetic radicals produced from TEMP can be used to quantify T cell activity by magnetic resonance imaging. In a B16-F10-OVA tumor model study, these fusogenic liposomes significantly promote the therapeutic efficacy of adaptive T cell therapy (tumor inhibition efficiency, 100.1 %).

CAR T cell therapy is another promising treatment for blood cancers, but it may cause safety concerns, such as 'on-target, off-tumor' cytotoxicity and cytokine release syndrome. Additionally, accurate dose control is challenging, especially in solid tumors. Researchers have developed light-switchable CAR (LiCAR) T cells that can be precisely activated to induce cancer cell killing using blue light optical dimerizers [15]. They have also optimized a surgically removable upconversion nanoplatform with blue light emission to regulate LiCAR T cells. Under near-infrared illumination, this nano-optogenetic immunomodulation platform shows a potent therapeutic effect on solid tumors (B16-OVA-hCD19) and lymphomas (Raji tumor) with greatly reduced side effects, suggesting that it enables precise spatiotemporal regulation of T cell-mediated immune response.

Outlook

Significant strides have been made in harnessing the power of nanomaterials to enhance cancer immunotherapy. Encouraging preclinical results underscore the urgent need for clinical trials to validate many of these concepts. However, a concerted effort to develop new, advanced materials is needed to realize the full therapeutic potential of these technologies.

To optimize the delivery of immunostimulatory drugs by nanoparticles into tumor tissues, a comprehensive approach is required to modulate the complex physiology of the tumor microenvironment. This involves normalizing tumor vasculature, degrading extracellular matrix, and increasing blood flow, which can enhance nanoparticle accumulation of therapeutic agents at tumor sites and generate a more favorable immune response. Intratumor injection requires nanomaterials that bind to the extracellular matrix or tumor cells to increase drug retention to the tumor and restrict the site of action to the local tumor, reducing the incidence of systemic immune-induced side effects.

To improve the therapeutic efficacy of cancer vaccines, intelligent nanomaterials that promote dendritic cell maturation and enhance cytosolic delivery of antigens are needed. Such nanomaterials not only promote MHC-I antigen presentation and prime $CD8^+$ cytotoxic T cells, but also simplify vaccine production by avoiding the need for additional adjuvants. Additionally, nanomaterials that directly interact with

tumor-eliminating immune cells, such as macrophages, cytotoxic T cells, and natural killer cells, should be explored further.

The stability, biodegradation pathways, and possible side effects of nanomaterials must also be carefully investigated during development. Moreover, product scale-up and clinical translation considerations, including experimental design, production, and regulatory issues, must be taken into account early in the development process [16]. These efforts will enable the realization of the full potential of nanomaterials in cancer immunotherapy and significantly enhance patient outcomes in the clinic.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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