Clinica Chimica Acta 492 (2019) 12–19

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Review

Advanced nanotechnology: An arsenal to enhance immunotherapy in fighting cancer

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ARTICLE INFO

Keywords:
Nanomedicine
Nanoparticle
Nanotechnology
Drug delivery
Immunotherapy
Micellar nanocomplex
Cancer
EGCG
Flavonoid

ABSTRACT

Cancer remains a major disease process with considerable healthcare and socioeconomic impact worldwide. Unfortunately, standard treatments using chemotherapy often do not effectively control cancer progression or prevent relapse. Over the past decades, the development of targeted therapies has substantially improved outcomes. Recently, immunotherapy has emerged as a new alternative for more effective cancer treatment and may even bring hope of a cure. Cancer immunotherapy functions by reinforcing a patient's immune defense system to fight the disease. Clinically, promising immunotherapy approaches have, however, been limited by unpredictable response and strong adverse effects. A drug delivery system (DDS) that effectively targets tumor and reduces drug exposure to normal tissue would mitigate these limitations. In this regard, nanotechnology has been intensively studied as a DDS for targeting tumors with various oncologic drugs. Several have resulted in improved treatment and outcome. Research has shown that nanoparticle drug delivery technologies can also be applied to immunotherapy. In this review, the current state of nanotechnology will be discussed. Because most cancer immunotherapies approved in recent years are protein drugs, this article will focus on a micellar nanocomplex (MNC) technology, a DDS platform especially suited for targeted delivery of these therapeutics to solid tumors.

List of abbreviations

DDS	drug delivery system
EPR	enhanced permeability and retention
MNC	micellar nanocomplex
EGCG	epigallocatechin gallate; (−)-epigallocatechin-3-O-gallate
Treg	regulatory T cell
MDSC	myeloid-derived suppressor cell
CAR-T	chimeric antigen receptor T-Cell
APC	antigen presenting cell
DC	dendritic cell
TAM	tumor-associated macrophage
TLR	toll-like receptor
TAA	tumor associated antigen

1. Introduction

Chemotherapy and radiotherapy are the conventional therapies for cancer. Patients undergoing these treatments typically suffer significant adverse effects and may face drug resistance as well as tumor relapse [1]. In the past decades, targeted therapies (primarily monoclonal antibodies targeting pre-defined tumor antigens) were developed to improve clinical outcomes either as monotherapies or in combination with chemotherapy or radiotherapy. Targeted therapies are more predictable and have less adverse effects in comparison to chemotherapy. In general, patients are more tolerant toward targeted therapies. For example, Herceptin treatment together with chemotherapy in breast cancer patients has better efficacy than chemotherapy alone. [2].

Over the past several years, immunotherapies have gained significant attention and a number of them have been approved for use globally. Ipilimumab, an Anti-CTLA-4 antibody for immune checkpoint blockade therapy was launched by Bristol-Myers Squibb (BMS) in 2011. This was followed by pembrolizumab, an Anti-PD-1 antibody developed by Merck which is a checkpoint inhibitor; and nivolumab, another anti-PD-1 antibody developed by BMS. Today, there are more than six immunotherapy drugs launched by various pharmaceutical companies worldwide.

Immunotherapy reinforces a patient's own immune system to effectively combat cancers [3]. However, immunotherapy suffers from limited Therapeutic Index (TI = efficacy/toxicity) because only small amount of the administered dosage can reach tumor while majority of

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https://doi.org/10.1016/j.cca.2019.01.027
Received 31 October 2018; Received in revised form 30 January 2019; Accepted 30 January 2019
Available online 31 January 2019
0009-8981/

the drug is distributed to other tissues in a patient. It often requires a high therapeutic dosage for tumor reduction. The high dosage can lead to systemic immune activations and induce severe adverse effects including cytokine storm, pneumonitis and colitis due to the interaction of immunotherapeutic agents with the host's own immune systems [4].

Various drug delivery system (DDS) technologies have been explored as tools to enhance the therapeutic index of immunotherapies for cancer [5]. The use of nanotechnologies has proven to be one successful approach for developing effective DDS. This method uses nanomaterials to encapsulate drugs and form nanoparticles which have certain physicochemical characteristics which serve to facilitate the successful drug delivery to targeted regions of the body. The advantages of using nanoparticles for cancer treatment include (1) stabilizing the drug molecules, (2) enhancing their solubility in blood circulation, (3) enhancing their biodistribution to specific tumor sites and (4) functioning as a slow-release agent. These features can increase the therapeutic efficacy and reduce adverse effects.

Several physicochemical parameters of nanoparticles including size, shape and surface charge are critical factors to consider in designing an effective DDS [6]. Particle size and surface structure determine their capability to deliver therapies at the targeted sites. Tumor lesions usually encompass larger interstitial space than normal tissues. Nanoparticles with a size larger than the interstitial space of normal tissues but smaller than interstitial space of tumor legions result in nonspecific (passive) accumulation of blood-borne nanoparticles in a tumor lesion. Surface structure guides specific (active) accumulation via molecular recognition. A ligand is often outwardly incorporated onto the nanoparticle to selectively target a tumor-specific receptor on a cell's surface.

In this review, the current status of nanoparticle technologies for anti-cancer drug delivery, especially for immunotherapy, will be discussed. We incorporate examples of the types of nanoparticles, the delivery principles, the active ingredients and the targets for cancer immunotherapy (Table 1). We also highlight the technology of Micellar Nano Complex (MNC technology). Utilized as a DDS technology for proteins, MNC has achieved outstanding preclinical results and has the potential to be an effective DDS that will enhance the Therapeutic Index of many immunotherapies.

2. Cancer immunotherapies

2.1. Background of cancer immunotherapy

Cancer is a complex disease caused by many different genetic alterations that control various cell functions. Today, the latest standards for cancer treatment emphasize the use of drug combinations to cover different genetic alterations and minimize overlapping toxicities. The strategy is to target a broad spectrum of cancer cells to improve overall tumor reduction and patient survival rate. In contrast, targeted therapies such as monoclonal antibodies, alone or in combination with other therapies, have shown significant clinical improvements that introduce the possibility of tailoring therapeutic strategy to individual patients [7]. Despite a positive initial response to treatment, tumor cells often establish diverse compensatory pathways that enable continued tumor differentiation and avoid cell death, thereby hindering the current standard treatments from achieving a sustainable tumor response or improved patient survival rates [1].

The human immune system has natural defense mechanism against cancer growth called “immune-surveillance”. However, the tumor microenvironment can often secret immune responsive factors like cytokines, chemokines and other metabolites to disrupt or escape these natural immune defense mechanisms. The disruption or escape mechanisms include ineffective presentation of tumor antigens (e.g., down regulation of MHC I), recruitment of immunosuppressive cells (e.g., Tregs, MDSCs), tumor release of immunosuppressive factors (eg, TGF-β, IDO, IL-10) and T-cell checkpoint dysregulation (eg, PD-1, CTLA-4) [8].

Rather than directly targeting the tumor, immunotherapy agents use the natural capability of patient's own immune system to fight cancer. Immunotherapy has been shown to effectively cure cancer patients by inhibiting the disruption of one's immune system induced by the tumor cells. Thus, many immunotherapy development strategies aim to activate co-stimulatory pathways to counteract tumor-mediated inhibition. Following this principle, several immune checkpoint blockade therapies have been approved including anti-CTLA-4 and anti-PD1/PD-L1 antibodies (Table 2).

Table 1
Examples of nanotechnology for anti-cancer therapy.

Platform	Advantages	Examples
Liposome	• The most characterized nanotechnology in medicine field. • The most investigated drug delivery system for tumor immunotherapy. • U.S. FDA has industrial guideline for liposome drug products.	Liposomal doxorubicin (Doxil) [36] Liposomal antigenic protein [39] PEGylated liposome-anchored combinatorial immunotherapy [40]
Polymeric micelles	• High drug loading ability and easy to modulate surface features. • Suitable for delivering chemical entities with poor water solubility	Thioguanine-loaded polymeric micelles [42] Micelles composed of poly(ethylene oxide)-*block*-poly- (α-carboxylate-ε-caprolactone) and STAT3 inhibitor [43] PSMA-targeted docetaxel nanoparticles [44] Aurora kinase inhibitor nanoparticles [45] T cell-targeting nanoparticles [46] Linear polyethyleneimine-based (PEI-based) nano-micelles [64] EGCG Micellar Nanocomplex [19,20,25]
Magnetic nanoparticle	• Magnetic Resonance Imaging property for theranostics application	Iron Oxide Nanoparticle carrying MHC-Ig dimer and anti-CD28 [56]
Nanoparticles with iron oxide core and zinc oxide shell	• Simultaneous use for delivering genes of interest with image agents	Multifunctional core–shell nanoparticle for dendritic cell-based cancer immunotherapy [57]
Inorganic nanoparticles	• Small size, large surface area and easy to be modified surface feature • Gold nanoparticles suitable for theranostics	Silica and gold nanoparticles [58,59] CuS nanoparticles [61]
Biomimetic nanoparticles	• Potentially lower toxic	Cancer cell membrane-coated nanoparticles [51] Viral protein [52,53]
Cholesterol-bearing pullulan -based hydrogel	• Self-assembly, relatively monodisperse and colloidally stable	Interleukin-12 nanogel [54]

Table 2
FDA-approved immune checkpoint inhibitors. The information is obtained from National Cancer Institute, Annual Plan and Budget Proposal Fiscal Year 2019, Developing Precision Immunotherapies.

Inhibitor	Target	Cancer type(s)
Atezolizumab (Tecentriq)	PD-L1	• Bladder cancer • Non-small cell lung cancer
Avelumab (Bavencio)	PD-L1	• Bladder cancer • Merkel cell carcinoma
Durvalumab (Imfinzi)	PD-L1	• Bladder cancer
Ipilimumab (Yervoy)	CTLA-4	• Melanoma
Nivolumab (Opdivo)	PD-1	• Bladder cancer • Head and neck cancer (squamous cell carcinoma) • Classical Hodgkin lymphoma • Melanoma • Mismatch repair deficient and microsatellite instability-high colorectal cancer • Non-small cell lung cancer • Renal cell (kidney) cancer
Pembrolizumab (Keytruda)	PD-1	• Bladder cancer • Head and neck cancer (squamous cell carcinoma) • Classical Hodgkin lymphoma • Melanoma • Mismatch repair deficient and microsatellite instability-high solid tumors • Non-small cell lung cancer

2.2. Cell-free therapies

Cancer immunotherapy can be categorized into cell-free and cell-based therapies. Cell-free therapy includes direct administration of therapeutic agents against tumor specific antigens and methods to facilitate host immune system to recognize and kill cancer cells. It can be conducted via different approaches such as using a tumor antigen, expressing an antigen gene, cytokine stimulation and blocking immune checkpoints to enhance the anti-cancer response of host T-cells (Fig. 1). Nanotechnology can substantially increase the efficacy of cell-free therapeutic agents and reduce toxicity [9].

2.3. Cell-based therapies

The cell-based therapy requires collecting and growing cancer-targeting immune cells from a patient, engineering the components to improve the immune functions of these cells, and then injecting the modified immune cells back into patients to more effectively destroy tumors. For example, genetically engineered immune cells such as chimeric antigen receptor T (CAR-T) cells have been extensively investigated for treating different types of cancer [10].

2.4. Immune checkpoint antibody

Immune checkpoints are regulators which protect cells from indiscriminate immune response. There are two general categories of immune checkpoint molecules. One is stimulatory checkpoint proteins such as tumor necrosis factor family CD27/CD40 and OX40 [11]. The other is inhibitory checkpoint proteins, such as CTLA-4, PD-1 and LAG3 [12]. Recently, the immune checkpoint molecules have been extensively studied as targets of cancer immunotherapy [13].

The programmed cell death-1 (PD-1) protein is expressed on immune cells including T- and B-cells whereas its ligand (PD-L1) is expressed on target cells such as cancer cells. The immune attack mediated by T-cells and B-cells against cancer cells is often dampened by the interaction between PD-1 and PD-L1 [13]. Another well characterized example of how cancer cells can escape immune control is the cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4). CTLA-4 is expressed on regulatory T-cells and interacts with CD80 or CD86 on antigen presenting cells (APC) to sequester CD80/CD86 from CD28 engagement, subsequently switching off the APC- and T-cell-mediated immune response [13]. In 2011, the first-in-class immune checkpoint blockade, anti-CTLA-4 antibody (Ipilimumab) was approved for commercialization in treating melanoma. More recently, additional antibodies against PD-1, Nivolumab and Pembrolizumab, were also approved for treating melanoma [12]. To date, six immune checkpoint antibody inhibitors have been approved globally for the treatment of eight types of cancers (Table 2). Currently, many clinical trials focusing on this kind of immunotherapy against a variety of different cancers are ongoing. Compared with conventional chemotherapy, these immunotherapies show better efficacy for end stage cancer patients [13].

Despite these advancements, significant gaps exist between efficacy

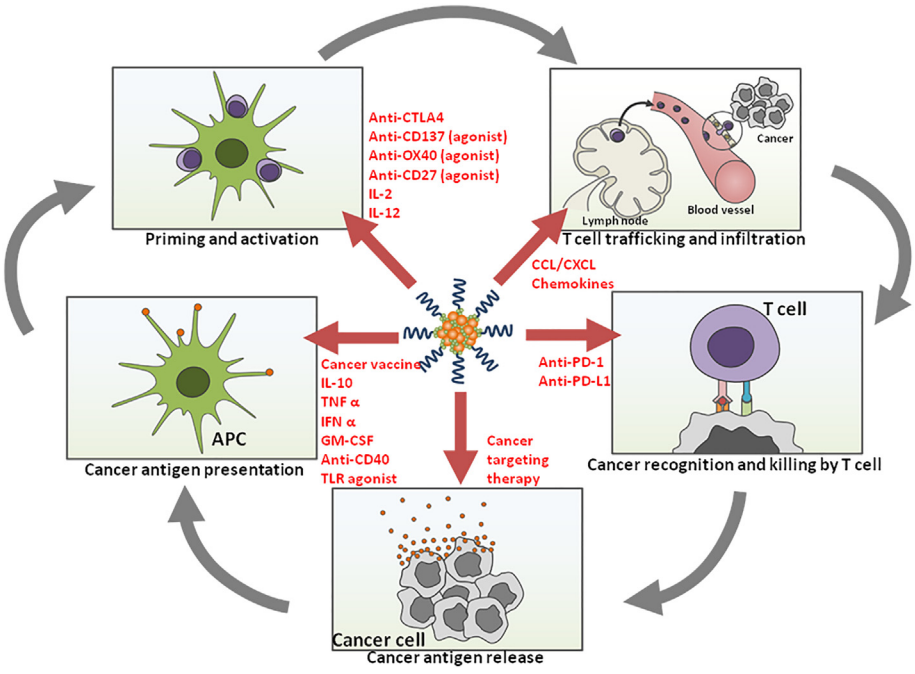

Fig. 1. Potential drug molecules for developing nanoparticle-based anticancer immunotherapy. Nanoparticles can be used to deliver (1) tumor antigens to antigen presenting cells (APC) as cancer vaccine; (2) antibodies to block immune checkpoint; (3) antibodies and cytokines/chemokines to boost anti-cancer immune response in multiple stages.

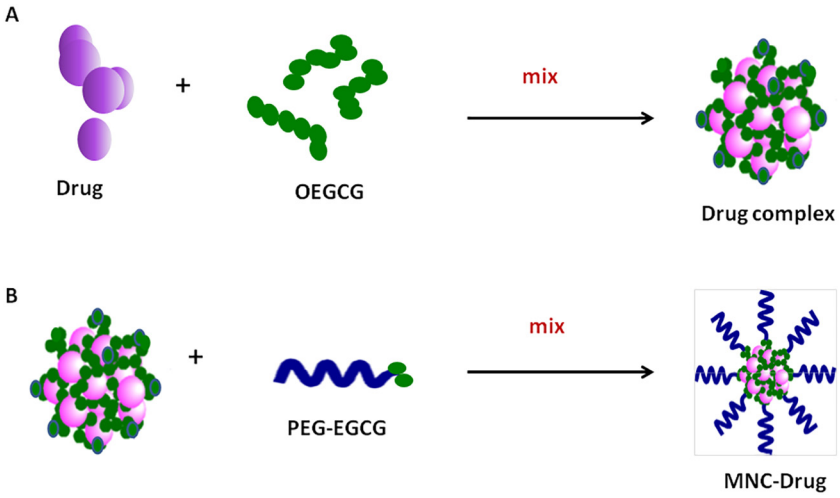

Fig. 2. Process of encapsulation of drugs to micellar nanocomplex (MNC).
(A) Drugs interact with oligomerized EGCG (OEGCG) via hydrophobic interaction to for the core structure. (B) PEGylated EGCG (PEG-EGCG) interacts with the core to form MNC-Drug complex with PEG outwardly integrated as the shell.

and safety because most of these therapies exert toxicity to patients due to systemic immune activation. To unlock their therapeutic potential, it is therefore necessary to improve the selectivity of these drugs in targeting the tumor microenvironment and reducing the exposure to normal tissues. A better immunotherapy strategy will be achieved by combining nanotechnology DDS with these developing therapies and gaining a deeper understanding of tumor immune response.

2.5. Cytokine-mediated immunotherapy and current difficulty

Immune modulators such as cytokines or other immune adjuvants have been proposed to boost the immune response to enhance cancer therapies. IL-12, a key member of cytokines, emerged in the mid-90s as a potent inducer of antitumor immunity based on initial encouraging results at the pre-clinical stage. However, systemic administration of IL-12 showed mixed anti-cancer results and demonstrated strong toxicities [14]. It can be hypothesized that sustainable, non-toxic anti-cancer responses with IL-12 may likely be achieved with a controlled and tumor-targeted delivery of this and other cytokines.

2.6. Cancer Vaccine

Delivering tumor-associated antigen (TAA) to antigen presenting cells has been proposed as an alternative way to trigger immune response against tumor [15]. This is also known as cancer vaccine. There are several types of cancer vaccines including synthetic protein, protein antigen, DNA and cell-based vaccines under investigation. For these cancer vaccines, accurately delivery to the targeted site is a critical goal waiting to be appropriately addressed.

3. Micellar nanocomplex (MNC) - drug delivery system (DDS) for cancer immunotherapy

Nanotechnology has been used successfully for small molecular chemotherapies [16,17]. For protein drugs, only a few types of polymer conjugations [16,17] such as PEGylation [18] have achieved some limited success. Many of the shortcomings are due to the fact that conjugation will alter the structure of a protein drug and often render it inactive.

MNC is a novel technology developed in recent years to significantly enhance the efficacy of anti-cancer immunoregulatory cytokine and monoclonal antibody targeted therapies in xenograft mouse model [19]. MNC technology does not use conjugation but hydrophobic interaction to retain complex drug molecules in nanoparticles. It does not change the molecular structure of protein drugs. After releasing the drug molecule at tumor tissues, the original bioactivity of the drug is restored. By maintaining MNC molecular size at 100–120 nm in diameter, MNC nanoparticles can effectively transport protein drugs from circulation to tumor site by enhanced permeability and retention (EPR) effect. This tumor accumulation due to EPR effect results in enhanced therapeutic efficacies for tumor reduction and reduced toxicity [19]. MNC technology can also be effectively applied to chemotherapies and gene therapies [20,21].

One critical consideration in designing suitable drug delivery system is the minimization of the toxicity induced by the delivery carriers themselves [22]. Carriers of many nanotechnology DDS are synthetic polymers with substantial toxicity concerns [23]. The base unit of an MNC carrier is (−)-epigallocatechin-3-O-gallate (EGCG). EGCG is an ingredient in many natural food items including green tea and apple and it has documented health benefits including anti-oxidation, anti-inflammatory and anti-infective benefits [24]. EGCG is in the family of polyphenol which interacts with proteins through hydrophobic interaction and is potentially able to protect protein drugs from proteolytic enzyme degradation during transportation to the tumor tissue.

MNC is comprised of two EGCG derivatives. The first is oligomerized EGCG (OEGCG), used to interact with proteins in the hydrophobic core [25]. The second derivative is PEGylated EGCG (PEG-EGCG) which creates the hydrophilic shell [19]. The MNCs can be manufactured through two sequential self-assembly steps including OEGCG and protein complexation for the core followed by encapsulation by PEG-EGCG (Fig. 2). This structure protects proteins from immunogenicity and degradation while providing a water-soluble carrier for drug delivery via the circulatory tract.

Evidence regarding MNC's capability to stabilize protein drugs in circulation has been demonstrated using several enzymes including xanthine oxidase, amylase and lysozyme, all of which interact with OEGCG via hydrophobic interactions. Activities of these enzymes were restrained when interacting with OEGCG and totally restored after dissociation from the complexes. This indicates the MNC carrier does not affect the bioactivities of the encapsulated drugs. The MNC has showed good stability in serum under 37 °C for over 15 days with no change in molecular size and shape of the nanoparticles. The encapsulated drug is also free from degradation by serum proteolytic enzymes in the blood. This suggests that protein integrity will be protected in the circulatory system.

MNC nanotechnology has been successfully applied to Trastuzumab

for the treatment of breast cancer. Compared with free Trastuzumab, MNC-Trastuzumab has much higher anti-cancer activity against HER2-overexpressed BT-474 breast cancer in xenografted nude mouse model. The biodistribution of MNC-Trastuzumab in mouse model showed that MNC-Trastuzumab had higher accumulation over free Trastuzumab in the tumor, and much lower accumulation in normal organs including the liver, kidney and lung. Remarkably, MNC-Trastuzumab showed a nearly 30-fold longer half-life than free Trastuzumab in blood, suggesting an excellent slow-release mechanism. This means a less frequent administration or lower dosing of the drug will be required to achieve effective treatments [19].

Interferon (IFN)-alpha 2a, an immunoregulatory cytokine in the cancer microenvironment has also been encapsulated with a MNC nanoparticle [19]. MNC-IFNα showed a stronger anti-cancer effect compared with free IFNα on HAK-1B liver cancer xenografted mouse model. Similarly, MNC-IFNα exhibited higher accumulation than free IFNα alone in the tumor over normal organs and a higher blood half-life similar to the data observed with MNC-Trastuzumab [19]. Together, these results demonstrated that MNC technology can deliver higher amount of administered protein drugs to tumor lesions with prolonged slow-release from blood circulation. These features reduce the off-target drug accumulation in normal tissues and results in lower toxicity.

Cancer immunotherapy agents including tumor antigens, antibodies and cytokines are all protein molecules (Fig. 1). IL-12 has proven to be a potent immunotherapy at the pre-clinical level but has failed to achieve significant outcomes in early clinical trials [14]. Recent literature indicates that a reasonable tumor-targeted IL-12 delivery system combined with radiation-, chemo- and immunotherapy may hold great promise against cancer [26]. MNC has the potential to be an ideal drug delivery system for the systemic administration of IL-12 as well as other cancer immunotherapies.

In addition, MNC has also been proven to exert good stabilization and delivery efficacy for doxorubicin [20]. Due to the structural similarity between EGCG and doxorubicin, strong intermolecular interaction was observed between these two compounds. The study also demonstrated superior tumor suppressive capacity over free doxorubicin and liposomal doxorubicin (Doxil), an approved liposome drug, in a HAK-1B liver cancer xenograft mouse model [20].

4. Nanotechnology used in cancer drug delivery

4.1. EPR effect

Nanotechnology platforms for tumor drug delivery often leverage the enhanced permeability and retention (EPR) effect, which means a molecule with a specific size range (usually 100–1000 nm) is preferentially accumulated in tumor tissue [27]. Two key features of tumor physiology contribute to this phenomenon. First, due to the high proliferation rate, tumor cells have higher nutrient demands. They usually secrete growth factors such as vascular endothelial growth factor (VEGF) to induce angiogenesis. This vascularization process forms new blood vessels with larger endothelial gap, which leads to an easier transport of macromolecules through the blood vessels compared to normal tissue. Second, the lymphatic vessel is less developed in a tumor region. This leads to suffocation of lymph circumfluence and contributes to nanoparticle accumulation. Together, these features cause nonspecific accumulation of nanoparticles in a tumor lesion.

The EPR effect of nanoparticle can effectively improve drug delivery. For example, porous silicon (PSi) nanoparticle has been used to reduce the metabolic rate and prolong the circulation time of loaded drugs [28]. Over the past two decades, many studies have shown the promising results of PSi particles compared with other drug delivery systems. The fabrication and application of PSi have been previously summarized by Li et al. [29]. Other nanoparticles such as self-assembled micelles, liposome and many other types of nanoparticles can increase the accumulation of desired drugs in tumor via EPR effect as well [Table 1] [30]. By design, MNC technology leverages this effect to target deliver encapsulated drugs to tumor tissues with very high efficiency.

4.2. Ligand targeting

For active (specific) accumulation, a ligand is typically attached to nanoparticles to deliver therapeutic drugs to specific cells with a matched receptor. This approach is used for cancer therapy in a case-by-case manner. Due to the complexity of target selection and ligand design, the mechanisms can vary significantly [30].

4.3. pH and temperature sensitive nanoparticles

In addition to the EPR effect, the characteristics of a tumor microenvironment can also be utilized for drug delivery. One of the most characterized metabolic alterations of cancer is elevated aerobic glycolysis due to the increase in nutrient demands [31]. This creates an acidic microenvironment in the tumor. Nanoparticles can be designed to release drugs at targeted sites triggered by the lower pH environment. For instance, Lee et al. showed that pH-sensitive P(Asp-g-Im)-PEG micelle with doxorubicin loaded can specifically release doxorubicin under pH < 7 condition due to the protonation and dissociation of the micelles in the tumor [32].

Ye et al. also developed pH-responsive micelles encapsulating doxorubicin [33]. The micelles are formed via self-assembly of poly(ethylene glycol)-*block*-poly(2-(diisopropylamino)-ethyl methacrylate) (PEGb-PDPA), and a vitamin E derivate (D-a-tocopheryl- polyethylene glycol 1000 succinate, TPGS). The doxorubicin is loaded and stabilized into the core of the micelle under pH 7.4. Once it is internalized by tumor cells, the acidic endosome environment causes the dissociation of the micelles and the drug is released.

In addition to pH value, temperature is also differentiated in tumor cells. Tumor tissues usually have higher temperature compared with the surrounding normal tissues. This feature can be used for applying temperature-responsive drug delivery system to cancer. For instance, Nanoparticle made of thermo-sensitive block copolymers of poly (ethylene glycol)-bpoly[N-(2-hydroxypropyl) methacrylamide dilactate] [mPEGb-p(HPMAm- Lac2)] has been developed [34]. Another example is Bi2S3 nanorods, which has good photothermal effect and was used for multispectral optoacoustic tomography (MSOT)/X-ray computed tomography (CT) [35].

4.4. Liposome

In 1995, the approval of liposomal doxorubicin (Doxil) opened a new chapter for nanomedicine [36]. Now U.S. FDA has industrial guideline for liposome drug products [37]. Several liposomal drugs have been approved and others are currently in clinical trials [38]. Based on our literature review, the encapsulated agents are all small chemical compounds such as chemotherapies or nucleotides such as siRNA.

Liposomal drugs for cancer immunotherapy are under development. One study used pH-sensitive polymer to modify the surface of liposomes to specifically release antigenic protein in the relatively acidic tumor microenvironment [39]. In addition, the same liposome contains cationic lipid which serves as an adjuvant [39]. By these modifications, the bioavailability and therapeutic efficacy of liposome drugs in triggering immune responses can be elevated.

Another study developed PEGylated liposome carrying anti-CD40 and CpG oligonucleotides. CpG oligonucleotide serves as a ligand for toll-like receptor (TLR) whereas anti-CD40 interacts with CD40. These immune-stimulatory ligands and antibodies with the PEGylated liposome can together prevent the immunotherapy from distributing to distal organs and increase the local concentration at the tumor lesion to enhance the T-cell-mediated anti-tumor response [40].

Currently, liposome-based therapeutic strategies are the most well investigated technologies in the field [26,27]. However, there is a lack of significant evidence showing that liposomes can work on protein drugs in-vivo. In addition to liposome, there are other nanotechnologies for drug delivery including natural polymers, synthetic polymers, micelles, dendrimers, iron-oxide nanoparticles, porous silica nanoparticles, gold nanoparticles and carbon nanoparticles [Table 1] [19]. These technologies will be elucidated in the following sections.

4.5. Polymeric micelles for cancer therapy

Polymeric micelles are self-assembled nanoparticles composing amphiphilic block copolymers which have been investigated for delivering cancer therapies [41]. The advantages of using polymeric micelles include their high drug loading capacity and easily modified surface features. They are especially suited for delivering chemical entities with poor water solubility to target tissues due to their amphiphilic characteristic. The hydrophobic core region interacts and stabilizes drugs whereas the hydrophilic shell region contributes to the solubility in blood and delivery to target sites. Polymeric micelles have been used to load 6-thioguanine to suppress the activity of myeloid-derived suppressor cells (MDSCs). In this way, the T-cell anti-cancer response is augmented in a murine model [42]. In another study, micelles composed of poly(ethylene oxide)-*block*-poly-(α-carboxylate-ε-caprolactone) and STAT3 inhibitor were conjugated to dampen the immunosuppressive effect in tumor cells [43].

Polymers poly(lactic-*co*-glycolic acid) (PLGA) and polyethylene glycol (PEG) have been used to assemble nanoparticles for delivering the chemotherapeutic agent docetaxel [44] and a targeted therapeutic agent Aurora kinase inhibitor [45]. More recently, similar technology has been applied for immunotherapy [46]. These nanoparticles were surface functionalized with fragmented antibody against CD8 to target T cell subpopulation with CD8receptor in blood and tumors. The study also explored conjugating PD-1 antibody to the nanoparticles with TGF inhibitor or Toll-like receptor (TLR7/8) agonist encapsulated. The results showed this system can increase the amount of tumor-infiltrating CD8+ T cells and enhance the therapeutic efficacy of PD-1 blockade.

4.6. Biodegradable and biomimetic nanoparticles

Biodegradable nanoparticles have advantages over non-biodegradable nanoparticles which may be immunogenic and able to induce toxicity or cancer [47]. Lipid or polymer-based nanoparticles are biodegradable and have been widely utilized for drug delivery [48] whereas other recent development initiatives have focused on the use of gold and silica nanoparticles [49,50].

Biomimetic nanoparticles, which mimic biological components, are considered to have lower toxicity and can also be applied to cancer immunotherapy. Cancer cell membrane-coated nanoparticles, which couple polymeric nanoparticle core with cancer cell-derived membrane and tumor-associated surface antigens, can be a way to develop a more effective generation of tumor vaccines [51].

Viral proteins also have strong potential for developing biomimetic particles. If the virulence can be reduced and engineered with functional antigens, these particles may safely and effectively induce anti-cancer immune responses. Studies have shown that the non-viral E2 subunit of pyruvate dehydrogenase can be used for encapsulating CpG and activating DCs [52,53]. Moreover, Cholesterol-beared pullulan, a hydrophobized polysaccharide, has been used as a nanoparticle to carry IL-12 for retarding tumor growth without serious toxicity in animal models [54].

4.7. Magnetic and inorganic nanoparticle

Magnetic nanoparticles have also been used for treating cancer. The major advantage of this type of nanoparticle is their applications in Magnetic Resonance Imaging (MRI) [55]. Within this category, superparamagnetic iron oxide nanoparticles (SPIONs) are widely investigated. Because they tend to aggregate, a biocompatible material can tentatively be used to reduce the aggregation. Dextran can coat magnetic nanoparticles to carry MHC-Ig dimer and anti-CD28 which enhance T-cell activity in the presence of a magnetic field which serves to localize the nanoparticles [56]. Using this approach, a larger number of activated antigen-specific T-cells will target the melanoma cells.

Nanoparticles with iron oxide core and zinc oxide shell are also used for delivering antigen to DCs. These nanoparticles can be used to deliver genes of interest and image agents simultaneously. Their specific accumulation in circulatory DCs has been observed [57].

4.8. Other inorganic nanoparticles

In addition to the abovementioned types of nanoparticles, other inorganic nanoparticles are potential options for cancer immunotherapy as well. The advantages of these inorganic nanoparticles include small size, large surface area, and easily modified surface features. For instance, studies have shown that silica and gold nanoparticles can be good carriers for cancer antigens targeting APCs for triggering anti-cancer immune responses [58,59]. With further modification of their surfaces with methods such as CpG conjugation, which can interact with toll-like receptor 9 (TLR9), they can be even more specific in targeting APCs [59].

Gold nanoparticles are suitable candidates for theranostics because they can generate good computed tomography (CT) imaging [60]. In this regard, the use of copper sulfide (CuS) nanoparticles has been also addressed. A study showed that combined with photothermal ablation therapy, CuS can be absorbed by DCs [61]. Specifically, the authors used hollow CuS nanoparticles carrying CpG oligonucleotide and found that upon laser irradiation, these hollow nanoparticles can be broken down into smaller nanocrystals which were efficiently absorbed by DCs. These results also suggest that combination therapy may serve to reduce adverse effects and increase efficacy.

4.9. Nanoparticles for RNAi delivery

RNA interference (RNAi) is a technology widely used for shutting down gene expression. Therefore, siRNA delivery using nanotechnology can increase the stability and targetability of siRNA for silencing a specific gene expression [62]. To achieve a successful siRNA delivery, many factors including optimized formulation for stability, route of administration, and ability to avoid adverse effects have to be considered [63].

Some studies using nanoparticle-mediated RNAi have shown their potential for cancer immunotherapy [62]. Cubillos-Ruiz et al. used linear polyethyleneimine-based (PEI-based) nano-micelles to encapsulate siRNA and found effective uptake by regulatory DCs with CD11c and PD-L1 expression in an ovarian cancer mouse model [64]. The DCs were transformed from immunosuppressive cells to APCs, subsequently promoting anti-cancer immunity via activating T-cells.

4.10. Nanoparticles for cancer vaccine

An appropriate nanoparticle delivery system will enhance the efficacy of therapeutic vaccines because they can efficiently deliver antigens to APC and sustain antigen release. An example is DepoVax (DPX-0907) which is under clinical trials for prostate, breast and ovarian cancers [65]. DepoVax is a liposome vaccine comprising several TAAs to target to DC, and clinical data demonstrate that DepoVax successfully activates cytotoxic T-cells against tumor at the site of injection [65].

4.11. Targeting circulating tumor immune cells

While EPR effect has been documented for nanoparticles to target

tumor cells via vascularization, methods for targeting hypoxia regions where blood vessels cannot reach have been less explored. Bryan et al. found that single-walled carbon nanotubes (SWNT) can be preferentially taken up by circulating monocytes, which is independent of EPR effect [66]. Furthermore, these monocytes will differentiate into tumor-associated macrophages (TAMs), which are able to penetrate through hypoxia regions in tumor. This study provides a strategy for enabling drugs to access previously inaccessible regions by conventional nanotechnology.

4.12. Targeting regulatory T (Treg) cells

Researchers have demonstrated that integrating monoclonal antibodies (mAb) to nanoparticles on the surface can guide the nanoparticles to directly target the cancer cells via antibody-antigen interaction [67]. It has also been reported that mAb can inhibit the function of regulatory T (Treg) cells. Because Treg is known to suppress the functions of other immune cells including nature killer (NK) cells and dendritic cells, the suppression of Treg function with mAb leads to cell death induction of cancer cells via enhanced immune response [67].

5. Summary

Cancer immunotherapy functions via blocking the pathways used by tumors to escape immune surveillance or by enhancing the reaction of immune cells against tumor. These cancer immunotherapies have shown promising results in multiple types of cancer. However, low efficacy and high toxicity in some patients are big challenges. An appropriate nanoparticle drug delivery system can unlock the therapeutic potential of these therapies by serving to reduce systemic cytotoxicity, increasing drug stability, and increasing the concentration of drug in targeted tumor lesions.

In this review, examples using nanoparticles to enhance anticancer immune response have been discussed. These studies use nanoparticle to target cancer or immune cells including (1) nanoparticle carrying immune-modulating ligand or antibody on its surface, (2) nanoparticle carrying small inhibitor or siRNA against immunosuppressive signaling and (3) nanoparticle carrying cancer vaccine (tumor associated antigen). However, clear evidence using nanoparticle to encapsulate antibodies of approved checkpoint inhibitors or immune-modulating cytokines is lacking. A major issue is the difficulty of controlling protein encapsulation and release by current nanotechnologies. By contrast, MNC technology described in this review has recently received close attention because it encapsulates proteins via non-covalent hydrophobic protein-EGCG interaction. Once MNC is delivered to the tumor site via EPR effect, it dissociates to release the drugs in their original molecular structure and, hence, maintain their full therapeutic capabilities. In the future, this or other similar technologies will potentiate the development of more effective and less toxic immunotherapies.

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