REVIEW

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Application of nanotechnology in circumventing immunotolerance

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Recent decades have witnessed a breakthrough in onco-immunology with cancer immunotherapy making a remarkable progress with promising therapeutic effects. Immunotherapy is a therapeutic approach that specifically attacks cancer cells by harnessing the host immune response. However, the existence of tumor immune escape and low specificity, limit the application of cancer immunotherapy. Nanocarriers with unique physiochemical properties are now being widely used for improving the anti-tumor effect of multiple cancer immunotherapeutic agents by offering alternate pharmacokinetics profile, site-specific delivery, and an enhanced cellular uptake. Nanocarriers can be engineered to target immunosuppressive tumor microenvironments to restore anti-tumor immune responses. In this review, we discuss the mechanisms of immune escape and how nanotechnology is applied to circumvent immunotolerance and improve anti-tumor immunotherapeutic effects. Perspectives on the rationale for designing nanocarriers-based cancer immunotherapy are also provided.

1. Introduction

Cancer is still one of the major life-threatening diseases and although great progress has been made in exploring the underlying causes, providing patients with durable anti-cancer resistance remains a major challenge. Cancer is a cell-autonomous, complex, and heterogeneous disease. Extracellular matrix, stromal cells in the tumor microenvironment, and infiltrating immune cells, can either inhibit or promote the occurrence, development, and invasion of diseases (Robert et al. 2015). Therefore, regulating the host immune system and the tumor microenvironment are particularly important in cancer treatment.

Immunotherapy is a new cancer treatment strategy which can inhibit cancer cells' outgrowth by employing the host immune system or relieving immunosuppression after surgical resection, chemotherapy, and radiotherapy. For some unresectable or metastatic tumors, immunotherapy can effectively inhibit tumor development, and enable the body to produce memory immunity, that inhibits proliferation and prevent recurrence (Topalian et al. 2011). However, due to tumor immune escape and the serious adverse reactions associated with immunotherapy, there are still many patients who do not benefit from immunotherapy (Topalian et al. 2012, Fesnak et al. 2016). How to expand this benefic to more patients is still a long-term aim for immunotherapy. Tumor immune escape is one of the major characteristics of tumor development and progression, in which the involved mechanisms are very complex, and include the participation of several genes, the metabolism, inflammation, blood vessels and other processes (Douglas et al. 2011). Therefore, clarifying the mechanism of immune escape is helpful in identifying new targets.

The rapid development of nanotechnology has a great impact on the field of tumor targeted therapies. In addition to their effects through passive targeting deliveries, that are mediated by enhanced permeabilities and retention (EPR), nanoparticles can be modified on their surface with tumor specific ligands which recognize and bind specific receptors, that are overexpressed on tumor cells, resulting in an active targeting delivery (Guo et al. 2015; Saha et al. 2013). Nanotechnology has also been widely used for tumor immunotherapy, including delivery of vaccines, antibodies, and immunomodulatory agents to specific immune cells, to improve their inhibitory effect on the tumor microenvironment (Hagan et al. 2018, Teo et al. 2015, Park et al. 2012). Researchers have developed a variety of nanocarrier delivery systems, with different properties, to improve the anti-tumor efficiency of immunotherapies. This article will review the mechanisms of tumor immune escape and the application of nanotechnology in tumor immunotherapy. Perspectives regarding the rationale for designing nanocarrier-based cancer immunotherapies are also provided.

2. Tumor immunotherapy and immune escape mechanisms

In clinical practice, the currently used immunotherapy mainly includes monoclonal antibody therapy and adoptive cell therapy (ACT). Monoclonal antibodies can block immune checkpoints on either T cells or tumor cells to restore T cell immune response against tumor cells (Krummel and Allison 1995). So far, the US Food and Drug Administration (FDA) approved immune checkpoint inhibitors for the treatment of patients with advanced melanoma and non-small cell cancer. These mainly include the monoclonal antibody drug ipilimumab (Quezada et al. 2006) a cytotoxic T lymphocyte antigen 4 (CTLA-4) blocker, monoclonal antibodies that block programmed death receptor 1 (PD-1) and PD-1 ligand (PD-L1), pembrolizumab (Rajan and Gulley 2014) and nivolumab (Borghaei et al. 2015). ACT is a therapeutic method of anti-tumor immunity based on transforming patients' immune cells *in vitro* to enhance their immune efficacy and that are infused back into patients (Gross et al. 1989). Tumor infiltrating lymphocytes (TILs) therapy is based on selecting and amplifying TILs that are extracted from the patient tumor *in vitro*. In clinical trials, this approach greatly improved therapeutic effects against melanoma (Rosenberg et al. 2011, Rosenberg and Restifo 2015, Lee et al. 2015). Chimeric antigen receptor T cell immunotherapy (CAR-T) is an approach that involves the transfer of a target extracellular antigen complex, derived from an antibody, to T cells (Thompson et al. 1994) or other immune cells (Bourquin et al. 2008). At present, the mainstream second-generation CARs can activate the

combination of the first signal (major histocompatibility complex) and the second signal (costimulatory molecule). Although the therapeutic effects on solid tumors is not ideal, it showed promising treatment outcomes in lymphoma (Curran et al. 2015) and B-cell leukemia clinical trials (Davila et al. 2012). However, these two types of therapies have obvious immune related side effects (Frey et al. 2014). Preclinical trials have shown that immune checkpoint blockade can cause systemic toxic reactions during intratumoral virus therapy and cryoablation (Kantoff et al. 2010) and severe immune related inflammatory reactions (Taneja 2012). Therefore, to meet the number and quality of cells required by the ACT therapy, therapeutic approach is unfortunately prone to induce immunotoxicity, and has a relatively long treatment cycle and high costs (Park et al. 2015; Lee et al. 2014; Maude et al. 2014).

The immune system distinguishes "self" from "non self" by recognizing specific antigens. The difference between tumor cells and normal cells relies on the expression of tumor associated antigens (TAAs) by tumor cells (Seremet et al. 2011). TAAs are mainly products of gene mutations or are proteins that have higher expression in tumor cells compared to normal cells. Although antigen-presenting cells can present TAAs and activate T cells to mediate a tumor-specific immune response, tumor cells can still develop a variety of immune escape mechanisms and evade the immune system, such as reducing the expression level of major histocompatibility complex class I molecules, reducing therefore T cells recognition and killing of target tumor cells; changing the expression level and identity of tumor-associated antigens which causes antigen drift; and expressing granzyme-specific serine proteases that inhibit perforin/granzyme killing effects (Bladergroen et al. 2002; Ray et al. 2012). Besides, tumor cells can escape immune response by inhibiting the function of immune cells. They can express the tumor necrosis factor related apoptosis-inducing ligand (TRAIL) to induce apoptosis of killer T lymphocytes, such as soluble Fas and Decoy receptor 3 (DcR3) (Töpfer et al. 2011; Ashkenazi 2002). Tumors' high expression of PD-L1 induces T cells apoptosis and anergy. Tumor cells can also inhibit the anti-tumor immune response by recruiting immunosuppressive cells, such as regulatory T cells, bone marrow-derived inhibitory cells and M2 macrophages, in tumor tissues which inhibits the anti-tumor immune response (Munn and Mellor 2006; Gadiot et al. 2011; Basu et al. 2006; Lee et al. 2009; Obermajer et al. 2011; Mantovani et al. 2004).

3. Application of nanotechnology in tumor immunotherapy

To further enhance the therapeutic effects of tumor immunotherapy, nanotechnology applications are subjects of intensive research. Researchers have developed numerous nanocarriers, with unique physiochemical properties, to deliver efficiently and specifically antigens or immunoregulatory agents, that regulate immune response. Nanocarrier-based immunotherapy has been widely used for cancer therapy and achieved promising outcomes in a variety of cancer types. The immunoregulatory agents can be immune adjuvants (such as polypeptides, proteins, nucleic acids, and oligonucleotides) and TAA and antibodies (targets) (Singh and Bhaskar 2014) (Fig. 1). Immune adjuvants and tumor associated antigens are the two most used components. One or more immunomodulators can be delivered to the target cells through a reasonable design of nanocarriers. The target cells can be divided into immune cells and tumor cells, depending on the specific mechanisms and the immunomodulator target. Because of the multi-functionalities of the nanocarrier in tumor immunotherapy, these can promote DC cells' recognition by target modification, and achieve a co-delivery of tumor antigens and immune adjuvants (Silva et al. 2013). In addition, nanocarriers can also solve some issues associated with free drugs' delivery systems, such as poor solubility, low bioavailability, and low therapeutic index. At present, a variety of nanocarrier systems have been developed in the field of tumor immunotherapy, including polymer-based nanoparticles and micelles, lipid-based liposomes, nanoemulsions and inorganic components of iron oxide nanoparticles.

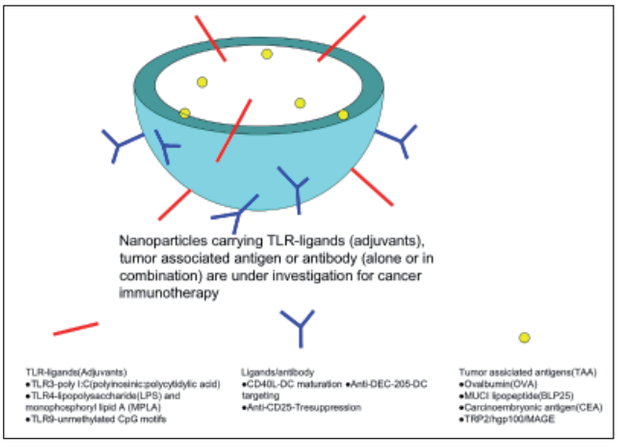

Fig. 1: Design strategy of nanocarriers in tumor immunology.

3.1. Polymeric nanoparticles

Polymer nanoparticles are a kind of solid colloidal particles, made of natural or synthetic polymer materials, that have a particle size of approximatively 10-100 nm, and that can be used as carriers of small molecule and biomacromolecule drugs (Panyam and Labhasetwar 2003). According to the different routes of administration, the particle size, shape, surface properties and degradation properties of the polymer can be appropriately adjusted. This kind of nanoparticles can effectively and stably encapsulate hydrophilic or hydrophobic drugs, protecting them therefore from degradation both *in vivo* and *in vitro*. Moreover, with an ideal particle size range, nanoparticles can easily pass a variety of biological barriers, which helps the drugs travel through the vascular endothelium and enter the target inflammatory or tumor site (Prokop and Davidson 2008). Moreover, the surface of nanoparticles can be appropriately modified to obtain ideal multifunctional nanoparticles (Edlund and Albertsson 2002) (Fig. 2).

Poly(lactic-co-glycolic acid) (PLGA) nanoparticles and poly (caprolactone) (PCL) nanoparticles have become the most widely used nanocarriers due to their outstanding biodegradability and biocompatibility (Danhier et al. 2012). In addition to their use as carriers, PLGA and PCL nanoparticles have been reported to have the potential as immune adjuvants, thus, they are also called particle adjuvants. On the one hand, PLGA or PCL nanoparticles, as carriers, can protect the immunogenicity of TAAs. On the other hand, they can also act as immune adjuvants to synergistically induce immune responses, including anti-tumor immune responses (Chen et al. 2014; Johansen et al. 2000).

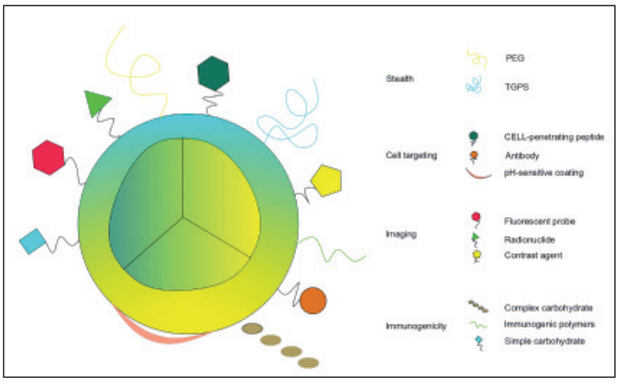

Fig. 2: Fabric of multifunctional nanocarriers.

Zhang et al. (2011) investigated then use of PLGA nanoparticles to co-load mouse melanoma antigen with Toll receptor-4 agonist monophosphorylate lipid A (MPLA). The results showed that the co-loading group can significantly inhibit the growth of melanoma cells, due to the synergistic effect of the melanoma

antigen and the immune adjuvant property of PLGA, and which led to the induction of an anti-melanoma immune response. Roy et al. (2013) co-loaded PLGA with the immunomodulator lipopolysaccharide (LPS) and chemotherapy drug paclitaxel (PTX), and the results showed that the average tumor volume of mice in the co-loading group markedly decreased compared to the mice treated with LPS or PTX alone. Additionally, the number of antigens presenting cells and T cells infiltrating the tumor site was significantly higher than that in the LPS or PTX alone group, which indicated that the co-loading of LPS and paclitaxel could promote tumor immune response and inhibit tumor growth in coordination with chemotherapy drugs. Schlosser et al. (2008) co-loaded ovalbumin (OVA) and CpG ODN into PLGA nanoparticles. The results showed that the number of antigen-specific $IFN^{-\gamma+}$ $CD8^+$ T cells, produced by a single immunization of mice with melanoma expressing ova, was 8 times higher than those of the control group, suggesting that co-loading of CpG ODN and tumor antigen in PLGA nanoparticles is a feasible approach to improve the therapeutic effect of tumor vaccines. In addition to the common PLGA nanoparticles, nanoparticles based on inorganic materials can also be used in tumor immunotherapy. Luis et al. (2010) used superparamagnetic iron oxide nanoparticles carrying fluorescent labeled antigens and showed that these nanoparticles could be used to analyze and quantify the uptake of nanoparticles by DC and monitor the distribution of antigens at the subcellular level, after uptake.

3.2. Polymeric micelles

Polymer micelles are thermodynamically stable colloidal systems of less than 100 nm in diameter that are spontaneously formed by amphiphilic block copolymers in a solvent system, at a suitable temperature and concentration (Torchilin 2001). Polymer micelles have a hydrophobic core and are often used to encapsulate poorly water-soluble or hydrophobic drugs. They can improve the bioavailability of the drugs and prevent their rapid degradation *in vivo* (Jhaveri and Torchilin 2014).

Polymer micelles also play an important role in tumor immunotherapy due to their small size, which allows them to easily infiltrate lymph nodes through lymphatic vessels. When they are used as carriers of anti-tumor vaccines, they are greatly beneficial in tumor antigen transfer or as immune adjuvants. Wilson et al. (2013) prepared micelles composed of dimethylaminoethyl methacrylate/pyridylmethyl thioacrylate, which were co-loaded with CpG ODN and tumor antigens. Compared with the free drug group, the co-loaded drug group promoted the cross presentation of antigens, which significantly improved anti-tumor immunity and body fluid immune response. Keller et al. (2014) prepared micelles composed of N-(2-hydroxypropyl) methylacrylamide/propylacrylamide/dimethylaminoethyl methacrylate/butyl methacrylate, which significantly improved the efficiency of DC cells antigens uptake and induced $CD8^+$ T cell response, indicating that polymeric micelles are potential tumor vaccines carriers.

3.3. Dendrimers

Dendrimers are hyperbranched nanocarriers formed by a central core, branched monomers, and functionalized terminal groups. They can be prepared by polymerization or divergent polymerization of branched monomers to produce hydrophilic surfaces and hydrophobic cores (Lee et al. 2005). The main physical and chemical characteristics of dendrimers are low viscosity, hyperbranched molecular topology, macromolecular size, and several chemically functionalized end groups (Yang et al. 2001). Today, the most widely studied dendrimer family is that of polyamidoamines (PAMAM), however, there are also poly(imide) and peptide dendrimers, such as poly(L-glutamic acid) dendrimers (Nanjwade et al. 2009). At present, several dendrimers have been used in clinical trials as vaccine vectors against breast cancer (Gilewski et al. 2007), small cell lung cancer (Krug et al. 2004) and prostate cancer (Slovin et al. 2003), and achieved good results.

3.4. Liposomes

Liposomes are a kind of lipid-like nanocarriers formed by a lipid bimolecular layer, with hydrophilic core at the center, and a particle size that usually ranges from 90 to150 nm (Conniot et al. 2014). A variety of drugs can be encapsulated in the water phase and lipid bimolecular layer of a liposome. Hydrophilic drugs can be encapsulated in the hydrophilic core, hydrophobic drugs can be encapsulated in the phospholipid bilayer, amphiphilic drugs can be located in the water phase and the phospholipid bilayer. Ligands and/or antibodies can also be modified on the surface of a liposome to endow it with a targeting ability. Previous studies have shown that liposomes have excellent properties (Aslan et al. 2013; Sharma et al. 2013; Sahoo and Labhasetwar 2003) and advantages, such as a slow and continuous drug release and a reduced drug toxicity through optimal drug distribution and pharmacokinetics (Sharma et al. 2013; Khan et al. 2008). Long circulating liposomes are the result of further optimization of ordinary liposomes, which usually refers to the modification of phosphatidylinositol, polyethylene glycol and ganglioside on the surface of liposomes, to prevent their adsorption by opsonin in the blood and clearance by the mononuclear phagocyte system, prolonging therefore their circulation time and the drugs action time in the body (Frank 1993; Krishnamachari et al. 2011) (Fig 3).

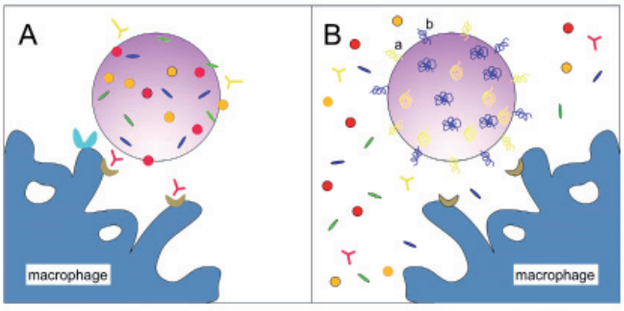

Fig. 3: Stealth liposomes to avoid internalization by the RES system.

Recently, several studies have used liposomes as nanocarriers for delivering or designing vaccines and anti-tumor drugs (Ewert et al. 2005). Zhang et al. (2017) modified liposomes with a pH sensitive transmembrane peptide and loaded them with immune adjuvant α-galactosylceramide, which significantly increased the uptake of DC cells and effectively activated NK-T cells. Yoshikawa et al. (2008) studied the *in vivo* and *in vitro* immune effects of tumor lysates carried by liposomes. Using lipids as carriers, the melanoma cell lysates that were transferred by membrane fusion, resulted in a significant increase in immune effects compared to those with non-melanoma cell lysates. Van Broekhoven et al. (2004) reported that an anti-DEC-205 antibody was modified on the surface of liposomes carrying antigens to enhance the targeting of the liposomes to DC cells. The results showed that the anti-tumor effect of the modified liposomes on the mouse model was significantly higher than that of the control group, and that the survival time of the mice in the treatment group was also significantly prolonged, indicating that modifications that target ligands enhances the targeting and uptake efficiency of the liposomes on DC cells, and further promoted the production of antigen-specific effector T cells. U'Ren et al. (2006) pointed out that cationic liposomes carrying plasmid DNAs can enhance anti-tumor immune response. Compared with the control group, the treatment group could induce the secretion of a large number of interleukin-12 (IL-12) and interferon-γ (IFN-γ), which rapidly activated $CD8^+$ T cells, and improved tumor immune efficiency.

3.5. Biomaterial scaffolds

Implanted polymer scaffolds which provide practical and functional advantages, similar to the traditional ACTs, can also be used to treat cancer. Traditional ACTs require the isolation of immune

cells (DC or T cells) *in vitro* and then re-transfusion to patients. Nanomaterials or hydrogel scaffolds implantation or injection produce patterned and customized local microenvironments that can comprise colocalized inflammatory factors, tumor antigens and immune signals (Li and Rudensky 2016). For example, by mice subcutaneous implantation of a porous nano PLGA scaffold, which combines granulocyte-macrophage colony-stimulating factor (GM-CSF) and CpG oligonucleotide (TLR9 agonist), can promote the recruitment and activation of DCs and eliminate local and remote tumors. It can also continuously carry out antigenic presentation and adjuvant signal transmission in the microenvironment and induce sustained immune responses (Ali et al. 2009). Mooney Group (Kim et al. 2015) designed an injectable self-assembled three-dimensional (3D) scaffold. The mesoporous nano rods (MSRs), with high aspect ratio, could self-assemble into a macroporous structure after subcutaneous injection in mice, and provide an ideal microenvironment for DC to flow into the lymph nodes and trigger immune responses (Fig. 4).

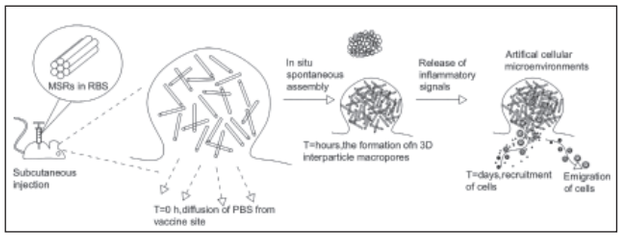

Fig. 4: Schematic representation of MSRs spontaneous assembly.

Because of the immunosuppressive tumor microenvironment, the effect of ACTs might be hindered. Thus, biomaterial scaffolds can be used to improve the therapeutic effect of ACTs when CARs are introduced into T cells. After tumor resection, the T cells in the tumor resection site can be continuously expanded and activated in the local area, which improve the antitumor effect (Stephan et al. 2015). Compared with the whole body or local injection of free T cells, T-cell scaffolds are also suitable for tumors, that partly removed, which lead to decreased residual cancer cells and cancer recurrence, and also provide conceptual evidence for targeted delivery cells, small molecules or biological agents.

For hematological malignancies, it is impossible to achieve a local microenvironment treatment by ACT stent. Nanotechnology can deliver drugs to T cells in blood circulation and directly *in vivo* without implanting a ACT stent, which not only optimizes ACT therapy, but also make it easier to transform. Stephan's Group (Stephan et al. 2010; Zheng et al. 2013) developed nanoparticles, containing drugs, monoclonal antibodies (mAbs), ligand antibody fragments or adjuvants, aimed at the surface of T cells, where they

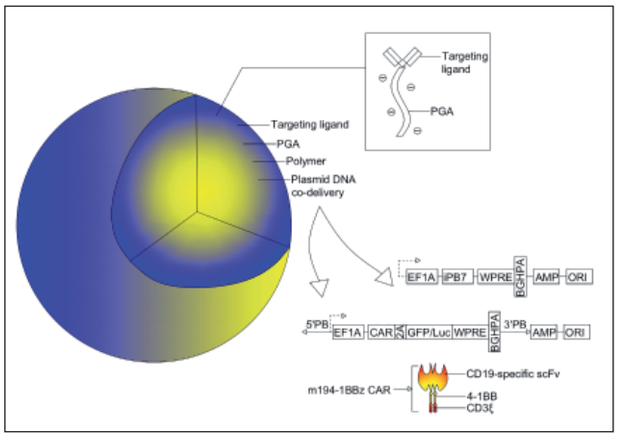

Fig. 5: Schematic representation of the T-cell-targeted DNA nanocarrier for lymphocyte-programming.

could provide continuous autocrine like stimulation signals, avoid the rapid decline of the activity and function of the transferred T cells, enhance the anti-lymphoma effect of ACT, and eliminate the distal tumor cells by inducing systemic immune regulation. This method is easy to operate and can be popularized as it can minimize adjuvants systemic toxicity without carrying out cell-free operations for each patient. On the other hand, due to the *in vitro* complexity and time-consuming amplification methods that are needed to produce a large number of tumor specific T cells, Smith et al. (2017) designed a method that could transform circulating T cells, enabling them to recognize tumors (Fig. 5). Using DNA loaded nanoparticles, to effectively introduce leukemia targeted CAR gene into the nuclei of mice T cells, and quickly edit the gene of T cells, resulted in 58 days increase of the leukemic mice average survival time, while avoiding complications. These polymer nanoparticles are stable, easy to store, have lower costs and are more suitable for wider applications than CAR-T therapy.

3.6. Nanocarriers for delivery of gene drugs

The delivery of small interfering RNA (siRNA) by nanocarriers provides a method for intracellular antigen synthesis, which has great potential in anti-tumor immunotherapy (Yin et al. 2014). Kranz et al. (2016) developed a lipid complex RNA lipoplex (LPX) loaded with a tumor antigen specific siRNA, which controls the transfection efficiency and spleen targeting by controlling the ratio of positive and negative charges. The outer fatty acid layer protects RNA from ribonuclease degradation and targets DC for RNA delivery. RNA-LPX mediates the effective uptake and expression of a DC and macrophage coding antigen, and can induce plasma like DCs and macrophages to release interferon (IFN) α. It was also found that RNA-LPX encoding new or endogenous antigens can induce strong responses of the effectors T lymphocytes and memory T lymphocytes. Li et al. (2016) constructed a cationic nanoparticle (NPsiCTLA-4) for delivering CTLA-4 siRNA, which could effectively transfer siRNA to T lymphocytes *in vitro*, and reduce the level of CTLA-4 mRNA and protein after T lymphocytes activation. *In vivo* experiments showed that the nanoparticles could transfer CTLA-4 siRNA into $CD4^+$ and $CD8^+$ T lymphocyte subsets present at the tumor site and reduced swelling. The proportion of tumor $CD8^+$ T lymphocytes and the proportion of regulatory T cells (Treg) in tumor infiltrating lymphocytes (TIL) were decreased, which enhanced the activation and anti-tumor immune response of tumor infiltrating T lymphocytes. NPsiCTLA-4 can effectively inhibit tumor growth and prolong the survival period of melanoma mice.

4. Mechanism of targeting immune cells with nanocarriers

Nanocarriers can be used to co-deliver anticancer drugs and immunomodulators, and to enhance the effect of tumor immunotherapy by alternating the pharmacokinetics profile, site-specific delivery, and cellular uptake.

4.1. Alternating the pharmacokinetics profile

Nanocarriers can increase drug solubility, change its absorption mode and distribution *in vivo*, which results in a change in the pharmacokinetic profile and prolongs action time to increase the antitumor effect. Colzani et al. (2018) encapsulated the monoclonal antibody trastuzumab (TZ), which targets the human epidermal growth factor receptor 2 (HER 2), into PLGA nanoparticles. The TZ nanocarriers prevented immune system clearance, achieved a controlled release and reduced adverse reactions. *In vitro* studies showed that the binding rate of HER 2 positive cells to fluorescence labeled TZ decreased by 92.2% 48 h after loading of the nanoparticles with TZ treated HER 2 positive cells, indicating that PLGA nanoparticles had an effective encapsulation and release effect on TZ. Compared with the unclosed TZ, the nanoparticles loaded with the antibody enhanced the degradation rate of HER2 through the lysosomal pathway, and reduced HER2 expression.

4.2. Site-specific delivery

Active targeting nanoparticles are usually achieved by modifying the bioactive ligands on their surface or periphery, which can be recognized and combined with overexpressed receptors in tissues or cells (Gao et al. 2014; Cheng et al. 2007). Surface modification is an important tool for achieving an active targeting of nanocarriers. In immunotherapy, such modification is also needed to achieve specific contact between nanocarriers and immune cells.

The DC cell surface expresses high levels of DEC-205, which can present exogenous antigen and MHC-I complex, thus promoting a highly specific cytotoxic immune response. Van Broekhoven et al. (2004) have modified the single chain antibody anti-DEC-205 on the surface of liposomes to achieve liposomes specifically targeting DC cells, and to effectively present the tumor antigen contained in the liposomes, and achieve a tumor specific immunotherapy effect. The mannose receptor on the surface of DC cells can also promote the internalization of nanocarriers through ligand receptor binding (Carrillo-Conde et al. 2011; Lu et al. 2007), enhance the further processing and presentation of antigens, and cause a stronger antigen-specific immune response. Other studies have shown that CD40 direct stimulation, on the surface of antigen-presenting cells, can activate antigen-presenting cells, enhance the presentation of tumor-related antigens, and stimulate the production of killer T cells, without relying on the co-stimulation signal, generated by the combination of the CD40 receptor on the surface of auxiliary T cells. It also provides a new approach in surface modification of nanocarriers that are used in tumor immunity (Vonderheide et al. 2013).

4.3. Enhancing cellular uptake

Cell uptake is a complex process that is affected by many factors, such as particle concentration, size, morphology, and surface modification. At present, it is believed that most nanoparticles enter the cell through endocytosis. Particles larger than 500 nm in diameter enter the cell through phagocytosis, while particles smaller than 500 nm in diameter enter the cell through pinocytosis. Small particle size nanocarriers can easily enter lymph nodes through lymphatic vessels, which increase DC cells uptake in lymph nodes, while large nanocarriers are more easily absorbed by phagocytes in the injection site or circulatory system. Positive nanocarriers are easily absorbed by DC cells, but their permeability in tissues is limited. On the contrary, negative, or neutral nanocarriers have a better permeability in tissues, but their affinity to DC cells is relatively low. The ability of rod-shaped or spherical nanocarriers to be absorbed by DC cells is stronger, while the ability of cylindrical and cubic nanocarriers is weaker (Singh et al. 2014).

5. Advantages and challenges of nanocarrier systems in tumor immunotherapy

Compared with traditional tumor therapy, the advantages of combining tumor immunotherapy with nanocarrier delivery systems lie in the following aspects: 1. Nanoparticles modified with targeting ligands can distinguish between target cells and normal cells, resulting in specific delivery of immunotherapeutic agents to target cells; 2. Tumor specific immune response can also have an inhibitory effect on metastasis; 3. Immune cells protect and prevent tumor recurrence.

However, various mechanisms of immune escape limit the therapeutic effects of tumor vaccines. For example, tumor cells can avoid CTL clearing effect by downregulating the expression of their own MHC molecules (Dunn et al. 2004). However, nanocarriers can effectively transfer immune adjuvants (polypeptides, proteins, nucleic acids, oligonucleotide chains) or tumor antigens to immune organs (lymph nodes or spleen) or antigen presenting cells through their unique physical and chemical properties, and achieve better immunotherapeutic effects.

Because of the presence of PAMPS on the surface of pathogens, these can be quickly recognized by the body's powerful immune system. Because the particle size of nanocarriers is similar to that of pathogens, they are often designed in the same way as simulating pathogens (Moon et al. 2012). For example, PAMPs are modified on the surface of nanocarriers to improve the efficiency of antigen delivery (Little 2012).

When the free form of the target antigen OVA is injected subcutaneously into mice, the amount of $CD8^+$ T is very low after cross presentation. However, after the antigen is encapsulated in PLGA nanoparticles, the antigen presentation of OVA can be significantly improved, $CD8^+$ T cells can be activated and the IL-2 secreted in the antigen group is 1000 times of that of the free antigen group (Shen et al. 2006). Other studies have shown that when the free tumor antigen is phagocytized by the antigen presenting cells, the amount of antigen presented *via* the MHC-I pathway is very small. They also showed that when the antigen presenting cells absorb the nanocarriers encapsulated tumor antigen, they can promote antigen presentation *via* the MHC-I pathway through cross presentation, and even detect the antigen presentation process 96 h after the completion of immunization, and induce the generation of a long time effective tumor specific CTL response (Harding and Song 1994).

In addition, nanocarriers can protect immunotherapeutic components (antigens/adjuvants) from degradation in the biological environment and improve the stability of antigens *in vivo*. Nanocarriers have also high drug loadings, and can release antigens slowly and continuously, which is helpful in improving the intensity of the immune response. Longer durations of antigen exposure can also induce an effective immune memory response (Waeckerle-Men et al. 2006).

However, there are still many problems and challenges in optimizing the synergy between immunotherapy and nanotechnology for clinical applications. The ideal nanocarrier should have the following characteristics: 1. Stable structure with no accumulation, and interaction with blood components; 2. It can protect the load from degradation in the blood circulation; 3. It has a good targeting abilities; 4. It can load a wide range of immunomodulators, and effectively transform the tumor microenvironment (Fridman et al. 2012).

To achieve the above functions, it is necessary to add multiple components to the nanoparticles, and large-scale preparation of these particles is challenging. Although many *in vivo* studies have shown encouraging anti-tumor effects, the clinical transformation of nano delivery systems for tumor immunotherapy, is still in its infancy, and faces great challenges. The current animal tumor models are too simple to simulate the complex human tumor microenvironment, and the nanoparticles that showed positive therapeutic effects may not induce similar effects in the human body. The immune system function of many cancer patients is greatly damaged, and their immune response may not be activated. The development trend to solve this problem is to add multiple components into the nano delivery systems and activate the immune response with multiple mechanism targets and at the same time, which may further increase the difficulty of producing complex multi-functional nanosystems. Therefore, the possibility of large-scale production should be considered in the design of nanosystem formulas (Sumatsu and Watanabe 2004).

On the other hand, the safety of nanocarriers for immunotherapy needs to be carefully evaluated. Some nanoparticles accumulate in tissues, such as the liver and spleen, and their interaction with blood components may enhance immunogenicity, leading to unnecessary immune response (Brentjens et al. 2003). Some nanoparticles can also cross the blood-brain barrier and lead to neurotoxicity (Swartz et al. 2012). Therefore, the selection of nanocarrier materials requires strict safety parameter evaluations.

Cancer immunotherapy based on nanotechnology covers drug intracellular transport, nanocarriers construction, and all required aspects for immune system interaction, imaging, and medical transformation (Grabbe et al. 2016, Mahjub et al. 2018), however, immune tolerance is often ignored. During immunotherapy, it may lead to issues of immune tolerance associated with the suppressive signal transforming growth factor-β produced by tumors, or CTLA-4 expressed by long-term activated T cells (Wolfram et al.

2015). Therefore, to avoid serious adverse reactions that directly target the development and clinical transformation of T-cells, it is necessary to use nanotechnology to effectively encapsulate immunostimulatory components and other drugs, adjust the combination of antigen-specific and non-antigen-specific immunostimulatory brake, specifically target and treat T-cells, and induce inflammation in the tumor to recruit immune cells, to eliminate immune tolerance.

In addition, how to make these nanodevices may be the biggest obstacle to clinical transformation. A controllable production can minimize the polydispersity of nanoparticles; therefore, it is essential to optimize the production process of the preparation. For example, an innovation in the field of 3D printing in regenerative medicine, is also of great significance in cancer immune engineering, which will make the location of specific cells, immune cells and cell matrix more accurate (Sharma and Sharma 2007), and which can be applied to the production of vaccines and scaffolds or to create implantable artificial three-level lymphoid structures with specific immune cells in the limited area (Zamboni et al. 2012).

6. Summary and prospect

To effectively improve the effect of tumor immunotherapy through nanotechnology, we need not only to understand how the immune system senses and responds to antigens and tumor threats, but also to deepen our understanding of the mechanisms of tumoral immune escape and the potential side effects of the anti-tumor immune response (Swartz and Lund 2012). In addition to the recognition of infiltrating cells and biochemical components in tumor microenvironment, the consideration of the tumor physical microenvironment and tumor draining lymph node (TDLN) should also be increased (Kanapathipillai et al. 2012). Extracellular matrix, as the physical medium of immunosuppression, prevents immune cells from infiltrating the tumor core (Zhu et al. 2016), while immune engineering can change the surrounding, the tumor extracellular matrix can change the physical microenvironment of a tumor (Gros et al. 2014) and promote the infiltration of immune cells. With the development of nanotechnology and the advances in immunology, there will be more breakthroughs in the cancer diagnosis, treatment, and prevention. Therefore, cancer immune engineering combined with nanotechnology is an emerging field.

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