Overview of tumor environment responsive nano-drug delivery systems in tumor immunotherapy

Yihao Zheng

1Department of biopharmacy, Nanjing University of Chinese Medicine, Nanjing, Jiangsu, 210023, China

* Corresponding author: author@e-mail.org

Abstract. Tumor immunotherapy is one of the most attractive fields and direction for scientific researchers due to its promising clinical efficacy. While the problems about the side effect and relatively low responsive rate for patients still remain to be solved. Nano-drug delivery system in anticancer therapy is playing important role gradually because of their specific characteristics, but there are still many biological barriers for drug-loaded nanocarriers. The tumor microenvironment and current application of nano-drug delivery systems in tumor immunotherapy are illustrated in this review.

1. INTRODUCTION

Owing to the deteriorating living environment and other endangered factors, the incidence of malignant tumors has been increasing in past few years and this situation happened almost in every region of the world, especially in low-income and middle-income countries, which now bear 80% of the worldwide burden of such diseases [1]. Given the situation of high mortality rates which related to the rapid development of cancer, the effective treatment of cancer has always been the attractive research field for clinics and scientific researchers.

The mainstream of tumor treatment methods is mainly divided into chemotherapy, surgery, radiotherapy and Immunotherapy. Chemotherapy is the mainstream cancer treatment modalities in clinic for various types of cancers. However, the severe side effects of chemotherapeutic drugs and inducible drug resistance make it difficult to meet ideal practical clinical requirements [2]. As a result, nanotechnology-based chemo-drug delivery systems have aroused widespread concern due to its potential benefit for treating cancer. Several therapeutic nanoparticles such as liposomes, albumin NP(Nab-paclitaxel), and polymeric micelles (Genexol-PM), have already been approved for clinical application for cancer treatment. At the same time, many other nanotechnology-based treatments are under clinical trials now [3]. Compared to other conventional drug administration, nano-drug delivery systems possess their own properties as fellows. Due to its drug loading capacity, it could increase the solubility of insoluble chemotherapy drugs; the release of loaded drug could be easily controlled with proper nanomaterials; in addition, nanocarriers could also help change the distribution characteristics of drugs in the body as well as the membrane transport mechanism of drugs.

The enhanced permeability and retention effect, also known as EPR effect, could be the crucial foundation which plays a core role in cancer nano-therapy. In most tumors tissues, the inner walls of tissues and blood vessels were damaged by rapidly growing tumor cells, causing a stronger permeability than that of normal tissues, selectively allowing polymer drugs with proper size to enter and remain in the tumor tissue, which enabled the polymer drugs targeting distribution ability. When
high molecular substances or nanoparticles (with a particle size of 10 to 500 nm) enter the tumor tissue, the damaged lymphatic circulatory system has no filtering effect on them, leading them retained near the tumor, slowly releasing the drug, and eventually improving the ability of drug targeting. Therefore, nano-based drug delivery systems would accumulate more in tumor tissues due to the EPR effect.

Although the EPR effect could somehow help drugs arrive at tumors, the complicated tumor microenvironment (TME) may become another barrier in the delivery process. When nanoparticles entered the tumor site, the highly reduction environment in tumor cells, the abnormal pH condition and the hypoxia both inside and outside the cell could also influence the drug release and the final treatment efficacy. That is to say, to design a microenvironment-responsive nano-drug delivery system is essential for the effective cancer treatment.

Immunotherapy has become the most attractive cancer treatment because of good clinical efficacy. In fact, there was a cycle of anti-tumor immunity in the body when tumor occurred. First, neoantigens formed by tumorigenesis were released and captured by dendritic cells. Subsequently, dendritic cells present processed antigens to T cells, initiating and activating effector T cell responses to cancer-specific antigens. The effector T cells migrate and infiltrate into the tumor bed and bind to the tumor cells through specific recognition, and eventually killed the target tumor cells. The death of tumor cells released more tumor-associated antigens and entered the next new tumor immune response cycle. However, imbalances and uncontrolled changes in each step made the tumor microenvironment changed, cytokines were generated and some inhibitory immune cells were recruited, assisting tumor immune escape and metastasis, finally making an inhibitory tumor immuno-microenvironment. Therefore, tumor immunotherapy is aimed at repairing these uncontrolled links, thereby reversing the tumor suppressive microenvironment and provide a better drug-delivery condition for nano-drug delivery system and achieving anti-tumor immunity.

This review is aimed to illustrate the tumor microenvironment and tumor-environment responsive nano-drug delivery systems in tumor immunotherapy.

2. TUMOR MICROENVIRONMENT
The abnormal physiological environment in tumors provided beneficial factors for cancer cells to proliferate and escape from death, and induced normal cells to into immunosuppressive differentiation. These would affect the occurrence, development, invasion and metastasis of tumors. Tumor microenvironment (TME) contained several parts, which jointly formed an inhibitory environment. Tumors were made up of cancer cells and many other types of relational cells such as fibroblasts, macrophages, lymphocytes, etc. Each cell type had its own function, but and cells were embedded in the extracellular matrix [4].

Tumor vessels surrounded, although were composed of endothelial cells, parietal cells and basement membranes, they blood vessels of cancerous tumors have various structural and functional abnormalities, some core areas even lack of endothelial cells or basement membranes. Abnormal organization of the tumor vessel structure caused twists and turns, making blood vessels to leak and create heterogeneous blood flow [5]. It was their spatial distribution that causing heterogeneous, swelling and tortuous structure, leaving avascular space of all sizes. Simultaneously, due to the rapid proliferation of tumor cells, the lymphatic vessels in the tumor tissue were incomplete, which leads to the leakage of substances from the blood vessels into the tumor tissue, and it was difficult to return from the lymphatic vessels.

In addition, high interstitial pressure existed around the tumor. There was no or collapse inside, causing too much liquid residues, so inefficient drainage was inevitable, so high interstitial pressure made tumor cells and related growth factors passed through peripheral lymphatic return tube and causes transfer; meanwhile, it was also difficult for the drug to spread internally in a normal manner.

Under steady-state conditions, the extracellular matrix (ECM) maintained the integrity of tissues and prevents malignant progression of tumor-prone cells by maintaining an overall healthy microenvironment. However, it changed when the condition was unstable. ECM remodelling caused collagen fibers to align, bind, and harden, which in turn changes the interaction between the matrix
and the matrix and tumor cells, thereby enhancing the proangiogenic secretion of a series of cells in the microenvironment as well as cancer cells. Therefore, this process promoted tumor cells invading the circulation from the primary site and recruited endothelial cells to promote tumor blood vessel formation and tumor growth, invade surrounding stroma, and finally metastasize.

2.1 Tumor biochemical environment
Compared with normal tissue, TME had several unique characteristics, such as mildly acidic intracellular pH, hypoxia which means the oxygen level can be pretty low and higher levels of certain enzymes [6].

The acidic pH was occurred mainly because the abnormal malignant proliferation of tumor cells, leading to insufficient oxygen assumption. Thus, cells began anaerobic breathing, causing the glycolysis of glucose, acidic products such as lactic acid accumulated in cells, correspondingly the pH decreased. It was the hydrogen ions that can be actively transported to the outside of the cells. The low blood vessel density around tumor tissue also lead to poor discharge and finally local accumulation of acidic metabolites. Simultaneously, the strong metabolism and rapid growth of tumors as well as abnormal peripheral blood vessels results in insufficient oxygen supply; oxygen consumption during this infinite proliferation process rocket up, leading to local hypoxia in the growth environment of tumor cells.

Normally, without the presence of protease, the rate of protein hydrolysis was quite slow. However, compared with normal cells, tumor cells, due to problems with the mechanism of controlling enzyme activity, the expression of enzymes cannot be controlled, which leads to significant differences between tumor cells and normal cells in expressing many enzymes.

proteases, glycosidase, and phospholipases, which were present in high concentrations, but were usually not present in healthy tissues. Matrix metalloproteinase (MMPs), especially MMP-2 and MMP-9, were well known to be involved in the invasion, progression and metastasis of many cancers. In general, MMPs were expressed at a low level. They were overexpressed during tissue remodelling, which can degrade the extracellular matrix and many other proteins, so it could affect the tumor microenvironment.

In cells, glutathione plays a decisive role in the cell's redox environment, the glutathione concentration inside and outside the cell was quite different. The glutathione concentration in the cytoplasm was as high as 1-10 mmol / L, while the extracellular glutathione concentration was only 1-10 μmol / L, some tumors even contain up to 7 times more glutathione (GSH) than normal tissues, thus presenting a reducing environment.

2.2 Tumor immune microenvironment
There were variable different immunosuppressive cells in the tumor microenvironment. Tumor-associated macrophages (TAMs) including M1 and M2 phenotype as well as the myeloid-derived suppressor cells (MDSC) and T-regulatory cells (Tregs) in the tumor microenvironment were explored [7].

TAMs were the most important types of leukocyte infiltration in the tumor microenvironment. They were present in almost all stages of human and mouse tumors, affecting tumor occurrence, development, invasion and metastasis.

TAMs were mainly produced by peripheral blood mononuclear cells, which were polarized to different phenotypes under the influence of the tumor microenvironment. TAMs infiltrated in tumor tissues could undergo polarizing formation and inhibit M2 macrophages under the influence of various immune factors. It secreted a variety of cytokines related to tumor growth, such as epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and chemokines CXCL and CCL family, etc., and could also produce TGF-β, IL-10, helping building the immune inhibitory response of tumor microenvironment.

MDSCs (myeloid-derived suppressed cell) are widely present in the spleen and tumor tissue, so an amplification and accumulation of MDSCs can be observed under pathological conditions, which
could inhibit the regulation of T cells and NK cells, leading to invasive tumor progression. In addition, MDSCs also inhibit the normal differentiation of dendritic cells by secreting high levels of heterogeneous cytokines, such as IL-10 and TGF-β, leading to the generation of immature dendritic cells, thus inducing the occurrence of Tregs.

Regulatory T cells (Tregs) are a subset of CD4+ T cells, it was reported that Tregs played their functions by inhibiting the proliferation and differentiation of normal T cells, then the antigen presentation is blocked. These may result in the negative regulation of tumor immunity.

3. CANCER IMMUNOTHERAPY

Cancer immunotherapy is on the rise and gradually plays important roles in clinical cancer treatment. To promote effective killing of cancer cells with the anti-cancer immune response, a series of gradual events must be initiated, which can continue and expand during the immune cycle, these events can be referred as cancer-immunity cycle [8]. Unfortunately, this mechanism seldom works due to the failures in the cancer-immunity cycle.

Firstly, neoantigens formed by tumorigenesis were released and captured by dendritic cells. Subsequently, dendritic cells (antigen presenting cells, APCs) were activated and presented processed antigens to T cells, initiating and activating effector T cell responses to cancer-specific antigens. The effector T cells migrated and infiltrated into the tumor bed, and eventually kill the target tumor cells through specific recognition to the tumor cells. The death of tumor cells would release more tumor-associated antigens and entered the next new tumor immune response cycle. Therefore, the key to anti-tumor immunity is exploiting the body's own immune cycle to fight against tumors.

However, when bearing cancer, most of the ideal anti-tumor immune circulation has imbalances in each step. For example, due to the immune editing of tumors, the neoantigens may become non-immunogenic, so they could escape capturing from dendritic cells, and DCs were not activated; moreover, the overexpression of some inhibitory factors such as indoleamine 2,3-dioxogenase (IDO) strongly affected the concentration of tryptophan which was essential to T cells, thus blocking the proliferation of effector T cells. in addition, the binding of PD-1 and PD-L1 in T cells and tumor cells would directly stop the killing process. Therefore, the goal of antitumor immunotherapy is to repairing one or more steps in the immunity cycle, such as inhibiting IDO and PD-1, thereby changing the tumor suppressive microenvironment and achieving anti-tumor efficacy.
3.1 IDO inhibitor
The IDO inhibitor can be the brand-new direction of cancer immunotherapy. As the main anti-tumor immune cells, the stable existence and proliferation of effector T cells are very crucial for the body's own anti-tumor immunity. Tryptophan is an essential amino acid in activating T cells and initiating immune cycle. However, in the tumor immune microenvironment, there is such an enzyme that contains heme, indoleamine 2,3-dioxygenase. In addition to its role in resisting excessive inflammation, it could inhibit the proliferation of T cells. It is the first step in the metabolism of tryptophan along the kynurenine pathway and the rate-limiting step of the entire process.

Studies have found that the kynurenine pathway is closely related to tumor immunity. The activity of this metabolic pathway led to the immunosuppressive effect of the tumor microenvironment. Deletion of the tumor suppressor gene BIN1 along with stimulation of interferon led to IDO overexpression in tumor sites. The reduction of tryptophan and the mounting of kynurenine may account for the suppression of effector T cells. Meanwhile, inhibitory regulatory T cells (Tregs) were also over-differentiated from naïve T cells, and the recruitment of myeloid derived suppressor cells (MDSCs) in tumor sites were also enhanced. Therefore, a suppressive immune microenvironment is induced. If IDO is inhibited, tryptophan will not be over-exhausted, thus increasing the number of T cells, in addition, reduce the number of suppressive Treg cells and MDSCs, thereby ameliorate the suppressive immune microenvironment in tumor tissues.

Currently, many IDO inhibitors have entered the clinical research stage, such as NLG919 and 1-MT.

3.2 PD-1 checkpoint inhibitor
Immune checkpoints are originally protective inhibitory pathways in the human immune system. They regulate the intensity and persistence of immune responses in peripheral tissues. Originally, they are used to prevent excessive activation of T cells and cause inflammatory damage. They play a "brake" in immune regulation. However, the tumor cells can over-express the immune checkpoint molecule PD-1 as to combined with the PD-L1 loci on the surface of T cells, so they can evade recognition and clearance of effector T cells, which enables their further grow and proliferation.

Based on this principle, a monoclonal antibody that co-suppresses a molecule (or a ligand) can be used to block its signal to achieve the purpose of activating T cells. The monoclonal antibody of PD-1: nivolumab and pembrolizumab has been approved by the FDA for non-small cell lung cancer and melanoma.

The mechanism of the antibody is to bind all PD-1 and kill these tumors by activating T cells. However, it cannot specifically activate tumor-specific T cell responses, which may bring some adverse reactions to patients.

4. NANO-DRUG DELIVERY SYSTEMS IN CANCER IMMUNOTHERAPY
Nano-drug delivery systems utilized nanometer-sized carriers which are designed for ameliorating the biodistribution of anticancer drugs. A variety of tumor-targeting nanomedicine now have been
developed. However, it was disappointing that despite the benefits mentioned before, current drug delivery systems only showed reducing toxicity but failed to improve treatment outcomes. For instance, Genexol-PM, a polymeric micelles composed of a diblock copolymer of monomethoxy PEGblock-poly(D, L-lactide) (mPEG-PDLLA), allowing a much higher paclitaxel dose to the tumor site without increasing the toxicity, but the higher doses still fail to generate better therapeutic efficacy [9].

The reason why nanomedicine failed to improve the efficacy could attribute to the obstacles when drug administration in the body. The aim of anticancer drug delivery is bringing drug to the tumor site, so free drug molecules could exert their pharmaceutical effect. Nanomedicine which went under intravenously administration must go through a link of five steps: circulation in the blood vessel; accumulation around tumor site through its leaky vasculature endothelial barriers; subsequent penetration deep into the tumor tissue to reach tumor cells; internalization into those cells; finally, the release of drugs. These processes were called the CAPIR cascade for short, realizing precise drug release in tumor sites [9].

However, due to the complicated microenvironment in vivo, it was almost impossible to pass through all these five steps perfectly for all kinds of drug delivery systems. Therefore, in order to maximize the final treating efficacy, it is important to make sure that each of which would not be completely impeded.

Firstly, when nanoparticles were injected into the body, it may circulate in the blood vessel, and the particles under 10 nm need to be filtered through the kidney; in addition, nanoparticles also could be enfolded by the plasma protein, causing opsonization and followed with elimination by the mononuclear phagocytic system (MPS). Moreover, the reticuloendothelial system (RES) specifically recognize the heterologous particles. Only nanoparticles with the size of 100 nm to 200 nm were reported to accumulate most in tumor sites. Therefore, nanoparticles with proper size and surface properties would ensure their circulation time and increase the possibility accumulated in the tumor sites.

The abnormal environment in tumor site (TME) also account for the limitation of the accumulation and distribution of nanoparticles in tumors. The extremely dense extracellular matrix and high interstitial fluid pressure make it difficult for nanoparticles to penetrate. Therefore, weakening the biochemical barrier was also important for effective drug delivery.

The cellular barrier could be the natural barrier to prevent the particles from entering the cells. Even if the nanoparticle evades the blockage of the cell membrane, the intracellular environment is so complicated that the nanoparticles can be easily captured by lysosomes and decomposed, resulting in the dissolution of the structure, the drug released quickly and remained uncontrolled. So, the rational response of nanoparticles in these special intracellular conditions would help drugs achieve better treatment efficacy.

Therefore, by rational design of nanomedicine to maximize the delivery rate in every step in CAPIR cascade, the safety and anticancer efficacy of nano-drug delivery could be better realized.

The stimulus-responsive DDS is an attractive therapeutic strategy which developed under the condition of special tumor microenvironment. It can preferentially deliver the nanomedicine to diseased cells with the nanoparticle which could sensitively responded by the abnormal pH, enzyme, reducing environment, and ROS in tumor cells [10].

The differences in pH in various organs or intracellular compartments, such as in pathological conditions like cancer, could be exploited in stimulus-responsive drug delivery systems design. Changes in pH can trigger drug release from the nanoparticles. When the pH reaches a certain threshold value, the charge type and quantity of the material molecules change, leading to the change of the electrostatic force between the material molecules. The pH-sensitive nanocarriers swell or de-swell, causing the particles to disperse or aggregate. For example, protonation of the amino group of chitosan (pKa * 6.3) induced the swelling of chitosan, leading to the release of TNF-α in tumor tissues with acidic environment [11]. In addition, differences in enzymes expressed between normal cells and tumor cells would also help nanocarriers response. Matrix metalloproteinase (MMPs) were over-
expressed in tumor cells, and it was reported that an MMP was used as a linker between a surface-bound PEG chain and TAT-functionalized liposomes. With active targeting ligand, the delivery system was delivered to the tumor sites and the enzymatic action cleaved the surface PEG chain, finally allowing the nanocarriers were internalized effectively into the cancer cells [12].

Due to the heterogeneity of the tumor cell itself, the efficacy of tumor immunotherapy is confined to a limited patient, with some obvious individual differences. Also, according to the distribution of immune cells around the tumor, the tumor immune microenvironment can be divided into different phenotypes: immune inflammatory phenotype which means CD4+ and CD8+ T cells can infiltrate in the tumor, while a large number of immune cells can be seen in the matrix; Immune exclusion table phenotype which is defined as immune cells cannot penetrate the parenchyma of the tumor and only exist in the stroma; Immune desert phenotype which refers to there are only a few T cells in the parenchyma or stroma of the tumor. Both immune exclusion phenotype and immune desert phenotype can be considered non-immune inflammatory phenotypes.

The limited efficiency of PD-1 inhibitor may probably attribute to the insufficient lymphocyte infiltration in tumor tissue, which refers to the non-immune inflammatory phenotypes. So, two strategies can be proposed to change the immune phenotype and enhance the efficacy of tumor immunotherapy: enhancing the immune system's response and the cytotoxicity against tumor cells; reducing related factors that were inhibitory to cytotoxic T cells killing tumor cells.

The first strategy means IDO inhibitor or PD-1 inhibitor can be used to enhance the costimulatory signals and pro-inflammatory cytokines of activated T cells so that it can improve the T cell survival environment and ensure the number and quality of T cells.

The second strategy can be referred as reducing the recruitment of myeloid-derived suppressor cells (MDSC) and the number of Treg cells; it can also change the polarity of TAMs which may result in the change or even the devastation of the state of CAFs;

Nano-drug delivery could combine these two strategies together, which means two strategies could work simultaneously by increasing the function of cytotoxic T cells as well as ameliorating the immunosuppressive microenvironment. It signified that a combination therapy using both chemotherapy and immunotherapy can be realized in order to integrate both of the advantages. Compared with conventional drug delivery system, it can well improve the medical effect of the immunomodulatory drugs with a much lower dosage than those of chemotherapy or anti-tumor targeting drugs such as kinase inhibitors, so accumulation of the drugs in every tumor cell can be avoided.

This is a commonly used method in clinical trials with the combination of IDO inhibitor drugs and immune checkpoint inhibitors like PD-1 inhibitor. At the ASCO2017 conference, Incyte announced that epacadostat combined with pembrolizumab for advanced NSCLC can achieve ORR, also, the disease control rate can reach to 35% and 63%, respectively. Patients with bladder and cervical cancer cohorts also achieved positive results [13].

Microneedle patch can be the medium when IDO inhibitor and PD-1 inhibitor were given into the patients. IDO inhibitors improved T cell numbers while PD-1 antibodies improve the quality of cytotoxic T cell. The advantage of using microneedle patch is that the drug can reach the stratum corneum and accumulate around the DC cells remaining in the skin around the tumor, thereby enhancing the local residence of the drug and reducing the leakage of the drug for systemic administration [14].

In current clinical trials, the combination of IDO inhibitor drugs with small molecule chemotherapeutic drugs has also been used.

The use of some small molecule chemotherapeutic drugs will release immunogenic antigens from dead tumor cells. Under appropriate conditions, the process of tumor cell death could activate the immune response; at the same time, the combined therapy could eliminate some of the regulatory T cells; moreover, due to the cytotoxic effect of chemotherapy drugs, a transient state of lymphopenia in the microenvironment has been induced due to the need for self-steady state recovery, which may lead to the establishment of the environment full of cytokines.
Professor Li’s team has been constructing such dual-functional carrier systems in recent years. The Fmoc domain of a drug response region was used to achieve the function of immunochemotherapy by physically encapsulating paclitaxel and chemically linking NLG919. It worked well in breast cancer tumor models in mice [15]. Also, this nanocarrier was also applied to the co-delivery of NLG919 and doxorubicin (DOX) when treating leukemia, which also exerted good antitumor efficacy and considerate safety [16]. Moreover, other researchers reported that the paratumor injection of PTX and 1-MT incorporated using thermogel also works in solid tumor via physical encapsulation of paclitaxel [17].

In addition, Subcutaneous tumors given 1-MT nanocrystals and oral paclitaxel nanoparticles possessed the best tumor-inhibiting effect compared to control groups [18].

5. CONCLUSION
As we know more about tumor environment, stimulus-responsive nano-based drug delivery system in cancer immunotherapy is going to be a promising research field, in the future, the combination application in chemodrug and immunotherapy and radiotherapy in nano-based delivery systems are worthy of more attention. In addition, the clinical translation of these drug delivery system should be an important further direction for cancer treatment.

Reference
[1] Bray F, Jemal A, Grey N, Ferlay J and Forman D. (2012) Global cancer transitions according to the Human Development Index (2008-2030): a population-based study. Lancet Oncology, 13: 790-801.
[2] Zhang R, Song X, Liang C, Yi X, Song G, Chao Y, Yang Y, Yang K, Feng L and Liu Z. Catalase-loaded cisplatin-prodrug-constructed liposomes to overcome tumor hypoxia for enhanced chemo-radiotherapy of cancer. Biomaterials: S0142961217303435.
[3] Shi J, Kantoff P W, Wooster R and Farokhzad O C. (2017) Cancer nanomedicine: progress, challenges and opportunities. Nat Rev Cancer, 17: 20-37.
[4] Dai Y, Xu C, Sun X and Chen X. Nanoparticle design strategies for enhanced anticancer therapy by exploiting the tumour microenvironment. Chemical Society Reviews: 10.1039.C6CS00592F.
[5] Jain R K and Stylianopoulos T. Delivering nanomedicine to solid tumors. Nature Reviews Clinical Oncology, 7: 653-664.
[6] Uthaman S, Huh K M and Park I K. (2018) Tumor microenvironment-responsive nanoparticles for cancer theragnostic applications. Biomater Res, 22: 22.
[7] Vasievich E A and Huang L. (2011) The suppressive tumor microenvironment: a challenge in cancer immunotherapy. Mol Pharm, 8: 635-41.
[8] Chen D S and Mellman I. (2013) Oncology meets immunology: the cancer-immunity cycle. Immunity, 39: 1-10.
[9] Sun Q, Zhou Z, Qiu N and Shen Y. (2017) Rational Design of Cancer Nanomedicine: Nanoproperty Integration and Synchronization. Adv Mater, 29.
[10] Ruttala H B, Ramasamy T, Madeshwaran T, Hiep T T, Kandasamy U, Oh K T, Choi H G, Yong C S and Kim J O. (2018) Emerging potential of stimulus-responsive nanosized anticancer drug delivery systems for systemic applications. Arch Pharm Res, 41: 111-129.
[11] Min K H, Kim J H, Bae S M, Shin H, Kim M S, Park S, Lee H, Park R W, Kim I S, Kim K, Kwon I C, Jeong S Y and Lee D S. (2010) Tumoral acidic pH-responsive MPEG-poly(betamino ester) polymeric micelles for cancer targeting therapy. J Control Release, 144: 259-66.
[12] Zhu L, Kate P and Torchilin V P. (2012) Matrix metalloprotease 2-responsive multifunctional liposomal nanocarrier for enhanced tumor targeting. ACS Nano, 6: 3491-8.
[13] Mautino M R, Link C J, Vahanian N N, Adams J T, Allen C V, Sharma M D, Johnson T S and Munn D. (2014) Abstract 5023: Synergistic antitumor effects of combinatorial immune
checkpoint inhibition with anti-PD-1/PD-L antibodies and the IDO pathway inhibitors NLG-919 and indoximod in the context of active immunotherapy. 74: 5023-5023.

[14] Ye Y, Wang J, Hu Q, Hochu G M, Xin H, Wang C and Gu Z. Synergistic Transcutaneous Immunotherapy Enhances Antitumor Immune Responses through Delivery of Checkpoint Inhibitors. Acs Nano: acsnano.6b04989.

[15] Chen Y, Xia R, Huang Y, Zhao W and Li S. (2016) An immunostimulatory dual-functional nanocarrier that improves cancer immunochemoetherapy. Nature Communications, 7: 13443.

[16] Sun J J, Chen Y C, Huang Y X, Zhao W C, Liu Y H, Venkataramanan R, Lu B F and Li S. (2017) Programmable co-delivery of the immune checkpoint inhibitor NLG919 and chemotherapeutic doxorubicin via a redox-responsive immunostimulatory polymeric prodrug carrier. Acta Pharmacol Sin, 38: 823-834.

[17] Ma H, Zhang Q, Yi L, Xu W and Fu C. (2017) Chemo-immunotherapy of spinal malignancy: paratumor injection of PTX/1MT-incorporated thermogel. Journal of Controlled Release, 259: e46.

[18] Calleja P, Irache J M, Zandueta C, Martinezoharriz C and Espuelas S. (2017) A combination of nanosystems for the delivery of cancer chemooimmunotherapeutic combinations: 1-methyltryptophan nanocrystals and paclitaxel nanoparticles. 126: 77.