Chapter 6

Antibody-Targeted Immunocarriers for Cancer Treatment

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Additional information is available at the end of the chapter

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Abstract

Nanocarrier's engineering based on fine chemical design and novel structural tailoring can provide practical solution to solve the problems in traditional cancer immunotherapy. Nanoimmunotherapy is thus defined as the application and further development of novel nanocarriers for enhancing immunotherapy. It has become one of the most intriguing fields due to its unique power in treatment and even cure of cancer since reported in last year. Herein, this chapter illustrates the state-of-the-art development in antibody engineering and cancer immunotherapy and gives an explanation why functional nanocarries including micelles and liposomes can be efficient for nanoimmunotherapy. We further illustrate how to promote the nanoimmunotherapy by the chemical design and carrier's engineering for the first time.

Keywords: Immunonanocarrier, antibody, nanoimmunotherapy, mAb engineering, drug delivery system

1. Introduction

Cancer can be caused by many elements, such as bacterial infection, radiation, and genetic abnormalities, and it is the leading cause of death all over the world. Nowadays, deaths caused by cancer are approximately one of eight of all deaths in the worldwide. Traditional cancer therapies such as chemotherapy, surgery, and radiation therapy have made a lot of progress in the treatment of cancer. However, they will still cause serious side effects or death by the damage of normal cells or organ including hepatotoxicity, cardiotoxicity, or nephrotoxicity. The application of nanotechnology in cancer treatment, monitoring, and control of cancer is

called “nanomedicine”, which is defined by the National Institutes of Health in USA. The cancer therapeutic index was significantly improved by the nanomedicines.

Compared with the traditional methods of therapy, newly developed cancer therapy based on the nanoparticles attracted extensive interest due to its unique advantages. However, there are still some drawbacks, such as the unfavorable in vivo performance for nanomedicine and the undesirable tumor escape in the immunotherapy. We know that in vivo performance strongly depended on the micelles structural properties; thus, the big gap between in vitro and in vivo can be overcome by micelles’ structural tailoring by chemical design and microstructural tuning. In addition, this fine micelles’ engineering can also provide practical solution to solve the problems in traditional cancer immunotherapy. In this chapter, we review the latest development in antibody engineering, nanomedicine, cancer therapy, and nanoimmunotherapy. We then give an explanation why fine micelles’ engineering with a special focus on the unique pathology of tumor microenvironments and properties of immunocells can obviously promote the in vivo performance and improve the therapeutic index of nanoimmunotherapy. In the chapter, we will take four parts to expound how the antibody-targeted immunomicelles play a role in cancer treatment.

2. Antibody engineering

Cancer-targeted therapy, aiming at targeting cancer cells and protecting normal tissue, is being developed rapidly and achieves significant improvement. One of the most significant advances in tumor-targeted therapy is nanomedicine, defined as the application and further development of nanotechnology to solve problems in medicine, specifically to diagnose, treat, and prevent diseases. [1, 2] Nanomedicines are designed to alter the pharmaceutical properties of loaded drugs (including pharmacokinetics (PK) and biodistribution (BD), or to function as drug reservoirs (i. e. , as sustained release systems), or both [3]). The adverse effects of conventional chemotherapeutics can be greatly ameliorated by nanomedicines. [4, 5] Also, the pharmacological properties of conventional drugs can be improved through the use of nanomedicines. [6, 7] On the other hand, nanomedicines can protect the drug from premature degradation and unfavorable interaction with the biological environment, improve the targeting to tumors by the “ enhanced permeability and retention (EPR) effect, ” and increase intracellular penetration. [8, 9] Recently, several nanomedicines have been approved or in clinical trials, such as Myocet (non-PEGylated doxorubicin liposomes), DaunoXome (daunorubicin liposomes), Onco TCS (vincristine liposomes), Doxil/Caelys (PEGylated doxorubicin liposomes), and Abraxane (albumin-bound paclitaxel nanoparticles).

Another significant advance in cancer-targeted therapy is the creation of monoclonal antibodies (mAbs) in cancer therapy. [10, 11] MAbs have been widely used alone or in combination with other chemotherapy agents in cancer therapy. [10-12] The use of mAbs in cancer therapy is growing rapidly due to their specific targeting to cancer cells and potent antitumor effects. [13] Until now, more than ten mAbs have been approved for cancer therapy. [14]

2.1. Monoclonal antibodies

MAbs are monospecific antibodies which are made by identical immune cells cloning from the unique parent cell. MAbs are typically made by fusing myeloma cells with spleen cells of mice immunized with antigens. The first generation of mAbs of murine origins is limited in clinic use owing to their strong immunogenicity and weak activity to elicit antitumor immune response. These defects are considerably overcome by the chimeric and humanized mAbs, which contain human Fc domains and retain targeting specificity by incorporating portions of the murine variable regions. Chimeric mAbs are generated by grafting the entire murine regions into the human IgG framework, whereas humanized mAbs are developed by grafting complementary-determining regions (CDRs) into the human IgG framework. [15-17] Recently, the fully human mAbs with little immunogenicity in humans are being developed rapidly using either phage display technology or transgenic mice. [18]

MAbs have achieved significant progress in cancer therapy. The most significant advances in the application of mAbs to oncology have been the approval of bevacizumab (Avastin, anti-VEGF antibody), cetuximab (Erbitux, anti-EGFR antibody), and trastuzumab (Herceptin, anti-HER2 antibody). Bevacizumab significantly prolongs the survival of patients with metastatic cancers of the colorectum, breast, and lungs, combined with standard chemotherapy regimens. [19] Cetuximab achieved potent antitumor responses in patients with chemotherapy-refractory colorectum cancer. [20] Herceptin has been shown to prolong the disease-free and overall survival of patients with breast cancer. [21]

2.2. Immunoglobulin-like antibodies

Immunoglobulin is a protein manufactured by plasma cells and lymphocytes and characteristic of these types of cells. Immunoglobulins play a key role in the body's immune system. Antibody, also known as an immunoglobulin, is a large Y-shaped protein used by the immune system to detect and neutralize foreign objects such as bacteria and viruses. The antibody recognizes a unique part of the foreign target, termed an antigen. Recent gene engineering could redesign the antibody by structure modification. After gene engineering, several antibodies still maintain their immunoglobulin-like structure but significantly enhanced the binding affinity or cytotoxic effects. Li et al. developed two genetically engineered tetravalent antibodies (TetraMcAb), respectively, derived from the anti-CD20 mAbs C2B8 and 2F2. [22] TetraMcAbs were not only effective in inducing complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC) against B-lymphoma cells as native divalent antibodies (DiMcAbs) but also had antiproliferative and apoptosis-inducing activity markedly superior to that of DiMcAbs. Wu et al. developed dual-specific and tetravalent immunoglobulin G (IgG)-like molecule-termed dual-variable-domain immunoglobulin (DVD-Ig)-that can be engineered from any two mAbs while preserving activities of the parental antibodies. [23] This molecule can be efficiently produced from mammalian cells and exhibits good physicochemical and pharmacokinetic properties. In an animal disease model, preclinical studies of a DVD-Ig protein demonstrate its potential for therapeutic application in human diseases.

2.3. Antibody fragments

In addition to mAbs and immunoglobulin-like antibodies, antibodies could also be reduced in size, dissected into minimal binding fragments, and rebuilt into multivalent high-avidity reagents. There are many kinds of antibody fragments. (i) The scFv is a fusion protein of the variable regions of the heavy (VH) and light chains (VL) of immunoglobulins, connected with a short peptide linker consisting of 10~25 amino acids. To provide flexibility and enhance the hydrophilicity of the peptide backbone, the most commonly used linker contains a combination of glycine and serine residues. The linker is usually rich in glycine for flexibility, as well as serine or threonine for solubility, and can either connect the N-terminus of the VH with the C-terminus of the VL. Despite of the removal of the constant regions and the introduction of the linker, this engineered antibody retains the specificity of the original immunoglobulin. (ii) Multivalent antibodies are constructed by multiple Fab or scFv. [24] (iii) Domain antibodies (dAbs), derived from the "heavy chain" of the immunoglobulins from camels, are the smallest known antigen-binding fragments of antibodies, ranging from 11 kDa to 15 kDa. They are the robust variable regions of the heavy and light chains of immunoglobulins (VH and VL, respectively). Due to the small size and inherent stability, dAbs are bioactive as monomers and can be formatted into larger molecules, which could be created with prolonged serum half-lives or other pharmacological activities. [25] (iv) Qiu et al. reported that the mimetics fused by two CDRs, VHCDR1 and VLCDR3, through a cognate framework region (VHFR2) retained the antigen recognition of their parent molecules and had a superior penetration capacity. [26] The antigen-recognition abilities of these B3 kDa mimetics surpass those of comparable fragments lacking the framework region. To our knowledge, these small antibody mimetics are the smallest antibodies among all the present antibodies.

3. Finely assembled micelles for promoting antitumor therapy

Almost 40% of newly discovered drugs have delivery problems due to their low solubility, permeability, and stability. In comparison with the traditional small molecule therapeutic agent, nanomedicine has offered new hope for detection, prevention, and treatment in cancer therapy because it extensively improves the solubility of poorly water-soluble drugs, [27] prolongs the half-life of drug systemic circulation, [28] releases drugs at a controlled rate, [29] delivers drugs in a targeted manner with little side effects, suppresses drug resistance, and reduces the immunogenicity. [30] Nanomedicine was generally not allowed to be used for the development of nanoscale or nanostructured materials to solve the problems in medicine via its unique medical effects. With the rapid advances in nanotechnology, many cancer therapeutic agents delivering systems have been developed based on nanoparticles, such as polymeric micelles, polymer-drug conjugates, dendrimers, liposomes, nanopolymer composition, and inorganic particulates with a size range of 1-1, 000 nm. Some of these products have been introduced into the pharmaceutical market. Doxil was the first liposomal drug formulation for the treatment of AIDS associated with Kaposi's sarcoma in 1995. [31] The polymer-drug conjugate, Abraxane, an albumin-bound paclitaxel drug formulation, was approved by

the Food and Drug Administration, USA (FDA) in 2005 as a second-line treatment for the breast cancer. [32-34]

However, some major challenges are raised as the clinical test of numerous ensuing nanomedicine products. The obvious drawbacks are the in vivo instability[35] and the fast clearance from the blood by the reticuloendothelial system (RES). [36] The most widely used strategy overcoming the instability is covering the carrier's with some hydrophilic polymers such as poly(ethylene glycol) (PEG) or poly(vinylalcohol) (PVA). Nanocarriers linked with highly hydrated flexible PEG successfully escaped from the RES. [37] The PVA coating also improved the particle's stability. However, it should be a commonsense that introducing too much adjuvant into the body resulted in the undesirable toxicity. Moreover, the size, structure, and surface electronic properties of the formulations were changed resulting in unfavorable therapy index. On the contrary, the micellar system mainly including the polymeric micelle and phospholipid micelle has successfully overcome the above drawbacks because these spherical nanoparticles have simple structure and no adjuvant. The lipid based micelles show high potential in the doxorubicin entrapping. [38] However, its intrinsic phospholipid structure resulted in the untunable micellar structure with D > 100 nm, which considerably limited the intratumor accumulation. Additionally, drug release from conventional liposome formulations is quite limited once these particles reach the tumor. [39]

Fortunately, the nanosized polymeric micelles (10-100 nm in diameter) self-assembled from amphiphilic block copolymers can significantly improve the hydrophobic drug solubility in the core via the similar-to-similar interaction. The micelle possesses well-defined hydrophobic core and hydrophilic corona structure in aqueous media. [40] On the other hand, the densely packed corona forming hydrophilic polymer chain can protect micellar system from the RES by reducing the interaction with serum proteins and renal filtration. [41] In comparison with lipid-based micelles, block copolymeric micelles provide a unique and powerful nanoplatform for anticancer drug delivery. The size of polymeric micelles can be easily tuned by varying the block lengths of the amphiphilic copolymer. It is also easy to modify micellar surface via the functional shell forming polymer. Both the tunable size range and the tailorable structure successfully reduce the renal filtration and obviously enhance tumor penetration. Some nanosized micelles such as PEG-PLA/PCL or PEG-PPO-PEG have significantly improved the in vitro /vivo application. Several polymeric micellar formulations are currently undergoing phase I/II clinical trials, which have shown significant antitumor efficacy and reduced systemic toxicity. [40, 41]

It is known that the endothelial cells of the tumor blood vessels proliferate at a 30- to 40-fold higher rate than those in normal tissues, which results in the larger endothelial cells gaps (200-700 nm, or sometimes even larger, up to 1. 2 μm) than 7 nm in the normal tissue. [42] Additionally, the high metabolism of tumor cells requires much more oxygen, nutrients, gas exchange, and waste removal. However, the heterogeneity structure and distribution of the tumor blood vessels as well as the blood capillaries slow down the energy exchange between intra- and extratumor. All these result in unique characteristics of tumor, that is, the unnormal tumor blood vessels with gap in 200-700 nm, [43] the relative high temperature of tumor (T > 37°C), [44] and the relative low pH (5~6). In order to further improve micellar delivering

profile, including the lesion's accumulating, cellular uptake, and intracellular release, many new stimulate-responsive micelles were extensively investigated with special focus on the tumor microenvironment. Utilizing the lower pH value in solid tumors and endosomes (5. 5), Kataoka's group explored the novel multifunctional pH-sensitive doxorubicin-conjugated PEG-p(Asp-Hyd-DOX) copolymer micelles. The pH linker broke as pH <6. 0 ensued a sustain release. [46] An enhanced accumulation in lung and colon tumors of the micelle-forming PEO-PAsp (ADR) conjugates after 24 h (ca. 10% dose per g tumor) was much higher than the free ADR (ca. 0. 90% dose per g tumor). Later, they further investigated the pH triggered intracellular release profile of poly(ethyleneglycol)-poly(aspartate hydrazone adriamycin) micelles and observed that the micelles can stably circulated in physiological conditions (pH 7. 4) and selectively release drug by sensing the intracellular low pH (pH 5-6). In vitro and in vivo studies show that the micelles had a good pH-triggered drug release capability, tumor-infiltrating permeability, and effective antitumor activity with extremely low toxicity. [45, 46] Okano's group used the temperature-sensitive poly(*N*-isopropylacrylamide) (PNIPAM) to investigate the cellular uptake of bovine carotid endothelial cells. As T>LCST, the cell uptake was significantly enhanced. In addition, the LCST of such PNIAPM can be tuned to ~39°C by introducing some hydrophilic monomer into the chain backbone. Thus, the system can shabbily circulate at 37°C but be disassociated as T approaching to 39°C. This PNIPAM was designed to enhance the intracellular release because the cargo structure was disrupted as phase transition. [47, 48] The oxidative condition in the extracellular medium and reductive conditions in the tumor was used to enhance intracellular release. For example, the bioreducible PEG-SS-P[Asp(DET)] micelles bearing the disulfide bridge showed both 1-3 orders of magnitude higher gene transfection efficiency and a more rapid onset of plasmid DNA release than micelles without disulide linkages. [49] Feng's group recently developed a micellar system containing a functional polymer of D-α-tocopheryl polyethylene polyethylene glycol succinate (Vitamin E TPGS or TPGS), which stabilized the micelle and further promotes synergistic effects with the encapsulated drug. [50] This is a novel micellar system. The formulation formed by folic acid-conjugated D-α-tocopheryl polyethylene glycol succinate 2000 (Vitamin E TPGS2k) micelles successfully suppress the tumor cell growth. [51] For improving the therapeutic effect, some other intelligent micellar systems such as light responsive poly(methacrylate) and poly(acrylic acid) (PAzoMA-PAA) micelle were developed. This trans-cis photoisomerization of the azobenzene group improved drug release. [52] In addition, the polymeric micelles conjugated tumor targeting V3lig-and cyclic-(arginine-glycine-aspartic acid-D-phenylalanine-lysine) (cRGDfK) to DOXO-loaded polyethylene glycol-polycaprolactone (PEG-PCL) micelles greatly enhanced internalization of the micelles through receptor-mediated endocytosis. [53]

These significant advances in intelligent block copolymer micelles have dawned upon a new era for nanomedicine. However, for translating an optimal micelle to clinical practice, there is still a big gap between in vitro and in vivo for lacking of understanding of the correlation between tumor unique characteristics (needs) and micellar physical chemistry properties (seeds). It is helpful to know that the micellar in vitro/vivo performance is strongly affected by its physical chemistry properties such as composition, dimension, microstructure, and the intelligent properties. The driving force for self-assembly is the strict solubility difference

between the hydrophobic and the hydrophilic blocks as described by the Flory-Huggins parameter.

4. Immunoliposome

Although liposomes have already achieved significant advance, antitumor activity could be further ehanced for liposomes through ligand-mediated targeting. For liposomes, the ligands would promote the selective binding and facilitate the intracellular delivery. The most commonly used ligands include mAbs or antibody fragments, folic acid, or receptor ligands. [54-59] MAbs or their derivatives (e. g. , Fab fragments, single-chain variable fragments (scFv)) are often adopted as the targeted ligands in LTLs. LTLs decorated with mAbs or their derivatives are termed as immunoliposomes. Immunoliposomes can be used to deliver various drugs, like chemotherapeutics, gene, or protein drugs, and significantly improve the therapeutic efficacy of conventional strategies in cancer. [60-64] When conjugated with antibodies as targeting ligands, immunoliposomes can target tumor cells with high specificity and affinity, resulting in significantly improved antitumor activity over untargeted liposomes. [65] The development of immunoliposomes, which perfectly combine antibody engineering and liposomes, is becoming a possible state-of-the-art in liposome research. This review discusses the recent development and therapeutic effect of immunoliposomes in cancer therapy. This review includes the following sections: antibody engineering, antibody conjugation strategies, therapeutic potential of immunoliposomes in cancer, challenges, and future perspectives of immunoliposomes.

4.1. Conjugation of thiolated antibody with liposomes

The most common conjugation strategy employs the reaction between thiol functions and maleimide groups, which form thioether bonds. This strategy consists of two steps. First, after reaction with traut's reagent (2-imiothiolane), the antibodies modified with free sulfhydryl are obtained. [66] Alternately, antibodies reacts with the heterobifunctional crosslinking agents such as *N*-succinimidyl 3-(2-pyridyldithio) propionate (SPDP) or succinimidyloxycarbonyl-α-methyl-α-(2-pyridyldithio) toluene (SMPT). Once modified with SPDP or SMPT, antibodies are treated with dithiothreitol (DTT) and form the free sulfhydryl. [67, 68] Also, antibodies react with the heterobifunctional crosslinking agents such as *N*-succinimidyl S-acetylthioacetate (SATA), S-acetylmercaptosuccinic anhydride (SAMSA), or succinimidyl acetylthiopropionate (SATP). [69-71] Once modified with SATA, SAMSA, or SATP, antibodies are treated with hydroxylamine and form free sulfhydryl. Second, thiolated antibody bearing the free sulfhydryl reacted with maleimide groups on the liposomes, and the resultant liposomes conjugated with antibodies were obtained. Attachment of antibodies to liposomes via a disulfide linkage A disulfide bond formed by two thiols is easily obtained. However, the disulfide bond is relatively unstable under reductive conditions. Thiolated antibodies could react with the pyridyldithio moiety of the anchor (PE-PDP) to form a disulfide linkage. This coupling strategy achieved efficient conjugation of antibodies to liposomes without denaturation of antibodies. [72-74]

4.2. Crosslinking between carboxylic acid on liposomes and the ligand

Antibodies could be conjugated to the liposomes by an amide bond using the membrane-anchored lipid functionalized with carboxylic acid end groups. This conjugation commonly used distearoyl-*N*-(3-carboxypropionoyl poly(ethylene glycol) succinyl) phosphatidylethanolamine (DSPE-PEG-COOH) offering carboxylic acid groups at the distant end of surface-grafted PEG chains as the membrane-anchored lipid. [75, 76] In the coupling reaction, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) and *N*-hydroxysulfosuccinimide (NHS) are usually used to form an acyl amino ester, which subsequently react with the primary amine of the ligand, yielding an amide bond. Suzuki et al. prepared liposomes conjugated with transferrin specific for transferrin receptor overexpressing solid colon 26 tumor cells, and the results indicated that the immunoliposomes recognized and bound specifically to target cells in vivo.

4.3. Antibody conjugation with Liposomes via a hydrazone bond

Antibodies can be covalently bound to hydrazide groups of the liposomes through their carbohydrate moieties to form a hydrazide bond. In the conjugation strategy, the carbohydrates groups on the constant region of the heavy chain of the antibody are oxidized by sodium periodate to produce reactive aldehydes, which form hydrazone linkages with the hydrazide groups on the PEG-terminus. [77, 78] It is has to be noted that the oxidation reaction should be performed in mild conditions to avoid the loss of antibody activity. The antibodies are correctly orientated on the surface of the liposomes because only the Fc region is involved in the conjugation reaction and the antigen binding site are protected. Furthermore, this conjugation strategy avoids the recognition of the immunoliposomes by the macrophages, resulting in longer circulation time of immunoliposomes.

4.4. Crosslinking between primary amines on liposomes with the antibody

Direct amine-amine crosslinking has also been investigated in antibody conjugation. [79] In the conjugation, two homobifunctional crosslinkers including glutaraldehyde and suberimidate are used, and no prior modification is required to add functional groups to the antibody. Briefly, the primary amine of phosphatidylethanolamine of the liposomes is firstly activated using the crosslinkers and subsequently conjugated to the antibody. It is reported that almost 60% of the antibodies were coupled to the liposomes, and these conjugated antibodies still retained their binding affinity. However, this conjugation strategy was rarely applied because the uncontrollable homopolymerization of antibodies or liposomes would happen during the crosslinking reaction.

4.5. Noncovalent methods for antibodies conjugated with liposomes

A noncovalent technique is an alternative means to for antibody conjugation to liposomes. The unique advantage of a noncovalent technique is easy and rapid performance without the need of aggressive reagents. For example, simply mixing antibodies and phospholipids during the preparation of the liposomes would achieve the binding of antibodies to the liposomes. [80]

However, the disadvantages of the noncovalent technique are obvious. The conjugation efficiency of antibodies is relatively low and liposome aggregation would happen. Furthermore, the amount of antibody conjugated to the liposome is not easily controllable, and the correct orientation of the antibodies is not guaranteed. Finally, conjugated antibodies are not stable and may detach easily. Thus, due to the weak interaction between the liposome and the antibodies, the noncovalent technique has not been widely used. However, it is noteworthy that one noncovalent technique, which uses the binding between streptavidin and biotin for attachment of antibodies to liposomes, is an exception. The binding of streptavidin to biotin is simple, highly stable, and reproducible; thus, the attachment of antibodies to liposomes using such a strategy is rather favorable and promising. [81] Generally, two strategies of antibody conjugation use the streptavidin-biotin interaction.

First, the streptavidin-modified antibodies were conjugated to the anchor lipid DSPE-PEG-biotin. The immunoliposomes redirected the biodistribution of entrapped drugs and showed specific targeting to the targeted organ overexpressing specific antigens, leading to significant accumulation in the targeted organ. [82] Second, the biotinylated antibodies were incubated with the targeted cells overexpressing specific antigens. Then streptavidin was added, followed by biotinylated liposomes. The results showed that the liposomes specifically bound to the targeted cells coated with antibodies but not to the control cells, which do not express the specific antigen. [83]

5. Well-defined nanocarrier's engineering for immunotherapy

Various immune cells such as B cells, T-lymphocytes (TL), and dendritic cells (DCs) are retained to the tumor. The modification of host immune system and/or the utilization of components of the immune system for cancer treatment are called immunotherapy, which mainly contains the active and passive form. Passive immunotherapy is to supply high amounts of effector molecules such as tumor-specific monoclonal antibodies (mAbs) to complement the immune system. Active immunotherapy is the utilization of humoral and/or cytotoxic T-cell effector mechanisms of the immune system following vaccination, namely, the cancer vaccines. This method can simultaneously activated antigen presenting cells (APCs), $CD4^+$ T cells, $CD8^+$ T cells, B cells, and innate immune cells, for example, granulocytes and NK cells. DCs are the most specialized and important APCs, which are responsible for an adaptive immune response. [84] Vaccines based on lipid-based nanocarriers can not only promote the accumulation in DCs in tumor-bearing hosts but also has a profound effect on DC function. [85] Poly(D, L-lactic acid-co-glycolic acid) (PLGA) nanoparticles carrying cancer-associated antigen (MUC1 mucin peptide: BLP25) and mouse-specific peripheral lymphocyte antigen (MPLA) obviously promoted native T-cell activation in normal and MUC1-transgenic mice. [86] The efficiency of vaccination strongly depends on tumor-specific antigens (TSAs) and vaccine delivery system. Polymeric nanoparticles attract extensive interest due to their facilely tunable composition, tailorable structure, unique intelligent properties, and high potential in cancer immunotherapy (i. e. , the nanoimmunotherapy).

Immunotherapy cannot only kill tumor cells in a specific manner but also alert the immune system to eradicate the disseminated tumor cells in blood circulation and micrometastases in distant organs. [87, 88] However, tumor cells can survive when they either maintain chronically or immunologically sculpt by immune "editors. " This well-known "immunoediting" refers to the elimination, equilibrium, and escape. [88] The new populations of tumor variants may eventually evade the immune system and escape from host immune surveillance by a variety of mechanisms including loss of MHC-I, adhesion molecules, tumor-associated antigens (TAAs), generation of regulatory T-(Treg-) lymphocyte, expansion of myeloid-derived suppressor cells ($CD11b^+$ $Gr\text{-}1^+$ cells, MDSCs), immunosuppression, blocking of NKG2D-mediated activation, and apoptosis induction of antitumor effector cells. [89, 90] Tumor-specific immune activation and nonspecific immune activation have been applied for overcoming such tumor escape. The tumor-specific immune responses are teaching the immune cells to recognize tumor cells specifically. B cells secrete antigen-specific antibodies that recognize, bind, and help to destroy the targets with the help of the $CD4^+$T cells. $CD4^+$ T cells recognize the antigens presented by MHC-II molecules and then stimulate B cells to produce antibodies to that specific antigen. Such antibody-coated cancer cells recognized and killed by NK cells, macrophage, and activated monocytes are called antibody-dependent cell-mediated cytotoxicity (ADCC). The nonspecific immune activation strategy mainly utilizes the cytokines (IL2 and IL8), the interferons (IFN-α, β, and IFN-γ), and the Toll-like receptors (TLRs) for trigging DC maturation, stimulating proliferation of $CD4^+$ and $CD8^+$ T cells and modulating the suppressive function of regulatory T cells (Treg cells). [91] Treg cells suppress TAA-specific immunity by inhibiting TAA-specific priming in tumor draining lymph nodes and further recruiting into the tumor microenvironment. [92] Thus, depletion, blocking, and tracking Treg cells in tumors or reducing their differentiation and suppressive mechanisms represent new strategies for cancer treatment. It was known that the knockdown of transcription factor Foxp3 gene in mature Treg cells resulted in the loss of their suppressive function. [93] However, the transfection efficiency is very low. The newly developed novel carbon nanotubes (CNTs) can enhance the transfection of Treg cells. [92] The PLGA nanoparticle (PLGA-NP) carrying murine melanoma antigenic peptides $hgp100_{25–33}$ and $TRP2_{180–188}$ can also induce cytotoxic T lymphocyte responses against tumor-associated self-antigens in C57BL/6 mouse. [94]

Thus, finely engineering nanocarriers from homopolymers, copolymers, and lipids with high loading and transferring efficiency, site-specific targeting to immune cells, high in vitro/vivo stability, and intelligent responsive to tumor microenvironment show high potential in nanoimmunotherapy. [95, 96] Tumor microenvironment is the main battlefield for tumor escape and immune system activation. As shown in Figure, the high proliferation and metabolism of tumor endothelial cells resulted in the unique properties of tumor microenvironment, including large endothelial cells gaps (200-1000 nm), the relative high temperature (T>37°C), low pH (5~6), lacking lymphatic nodes, and lymph vessels. [97] This unique pathological condition of microenvironment offers challenges for novel nanocarrier's engineering. Based on the self-assembly mechanism, well-defined micelle and vesicle with surface targeting decorating were finely engineered. [98] We found that the temperature-regulated passive and mAb-tuned active dual targeting immunomicelles significantly enhanced

intratumor accumulation and cellular uptake. The nanostructure and the dimension were also tailored to match the large endothelial cells gaps in tumors with enhanced permeability and retention (EPR). [86] The extracellular pH is ~7. 4, but the pH in the endosome and microenvironment is ~6. 0. This value is still lowered to ~5. 0 in the lysosome. The hydrolysis rates of polyester such as polylactic acid, polyglycolic acid, and their copolymers can thus be tuned for endosomal and/or lysosomal delivery. [99] Additionally, the endosome is reductive, but the lysosomal is oxidative. This difference is very important for spatial delivery antigens for MHC presentation. The antigens for MHC class I pathways must be available in cytosol, whereas those for MHC class II molecules must be present in endolysosome. The finely engineered lipids with protein antigens in nanovesicle core and lipid-based immunostimulatory molecules in the walls successfully elicits endogenous T cell and antibody responses, which showed rapid release adjuvants in the presence of endolysosomal lipases. [100] Some danger signals (adjuvants) for APC activation are present on the plasma membrane. So nanocarriers engineered from polycations such as polyethyleneimine (PEI) or its graft copolymers (Figure) hold favorable effect on membrane destabilization by the "proton-sponge" effect, which can also control the endosomal release. [101] Both structural defects and fibrosis of the interstitial matrix result in poor/dysfunctional T-cell priming in tumor microenvironment. However, the forced expression of the tumor necrosis factor (TNF) can induce naive T-cell priming. Thus, delivery stimulator such asCD80, interleukin-4 (IL-4), and cytokines by intelligent nanocarriers to tumor microenvironment can produce T-cell priming with the microenvironment reversion. [102]

DCs appear in most peripheral tissues where antigens typically first encounter the immune system. Immature DCs phagocytose the encountered antigens followed by the activation, maturation, and migration to draining lymph nodes. They present antigens to their cognate naive T-cell partners and instruct the anergy, tolerance, or immunity. Then the antigen-specific T-cell immunity is initiated. Noted here is the timing at which antigen and adjuvant reach DCs is crucial. If the maturation stimulus is too late, tolerance will be induced. If the antigens reach mature DCs, they will not be efficiently presented. The intelligent responsive polymer carriers can be finely designed to regulate the antigen's communication with DCs. Some lipids had successfully been used to promote the lymphatic trafficking and endue the DCs mutation. [103] The DCs preferentially take up smaller particles with size similar to viral (~20 nm), whereas macrophages ingest the large particles with size around bacterial. It is also worth mentioning that PLGA-NPs (500 nm) are more effective than microparticles (~2 nm) in stimulating CTL responses. The DC's phagocytosis is also affected by nanoparticle's surface charge. [104] Cationic particles are particularly effective for uptake by DCs and macrophages due to that the ionic attraction increases the particle binding and internalization. Above-mentioned nanocarrier's size, microstructure, charge, and intelligent properties can be facilely engineered by tuning polymer composition and particle formation process. In addition, specific DC-specific antibodies such as anti-CD11c and anti-DEC205 can enhance nanocarrier's accumulation in DCs. The PLA nanoparticles loaded dacarbazine (DTIC) decorated with TRAIL-receptor2 (DR5) mAb (DTIC-NPs-DR5) showed high internalization by DR5-overexpressing metastatic melanoma and chemo-immunocooperative therapeutic effects. [105] Based on our understanding of the molecular mechanism of immunoescape and the physiologic

conditions of tumor, the nanocarriers in nanoimmunotherapy should be further finely engineered with well-defined dimension, intelligent properties, specific targeting, advance lymphatic imaging, and precisely intracellular release for optimizing the therapeutic index.

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