Nanomedicines Targeting the Tumor Microenvironment

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Abstract: We review recent progress in cancer nanomedicine to overcome the delivery barriers in tumor microenvironment, including the understanding in the nanomedicine delivery process, stimulus-responsive delivery, and several new strategies to normalize tumor microenvironment. The application of nanomedicine in cancer immunotherapy, a renewed cancer therapy by recent breakthroughs, is also highlighted.

Key Words: Cancer immunology, extracellular matrix, nanomedicine, nanoparticle, near-infrared light, photothermal, stimulus-responsive drug delivery, triggered drug delivery, tumor microenvironment, tumor penetration


Cancer nanomedicine refers to the application of nanotechnology-based therapeutics and imaging agents for the diagnosis, monitoring, prevention, and treatment of cancer.1 Cancer nanomedicine is expected to change the oncology by delivering a wide range of payloads with favorable pharmacokinetic properties, capitalizing on molecular targeting for enhanced specificity, efficacy, and therefore safety. Tumor vasculature is typically permissive for transvascular transport of nanomaterial sizes less than 100 nm, and lymphatic dysfunction in tumor causes poor clearance of nanomaterials—both allow for enhanced permeation and retention (EPR) of NPs into tumor.2 The hyperpermeability of tumor vessels’ and dysfunctional tumor lymphatic vessels has been observed in human patients’ tumors, suggesting the existence of the EPR effect in human tumors. The EPR effect has been demonstrated to be the key pharmacokinetic feature for passive tumor targeting and reduced systemic toxicity with cancer nanomedicines.3

Many nanomaterials have been used as drug delivery vehicles and/or imaging agents. They include liposomes; polymer carriers, such as micelles, hydrogels, dendrimers, and nanofibers; metallic nanoparticles (NPs) (gold, silver, etc.); carbon nanostructures (nanotubes, graphene, etc.); inorganic NPs, such as silica NP; and hybrid nanomaterials.4 Different classes of nanomaterials with unique properties are optimal for specific applications. For example, the incorporation of chemothapeutic agents in liposomal or polymeric NP delivery vehicles has exhibited improved drug solubility, reduced drug clearance, reduced drug resistance, and enhanced therapeutic effectiveness.5,6 Several NP therapeutics, for example, Doxil (~100-nm PEGylated liposome loaded with doxorubicin) and Abraxane (~130-nm albumin-bound paclitaxel NPs), have been approved by the US Food and Drug Administration and have shown improved pharmacokinetics and reduced adverse effects compared with their parent drugs.7,8 In addition, metallic particles are promising therapeutic agents that convert light to heat (photothermal effect) to kill cancer cells, with clinical trials in head and neck cancer and lung cancers.9 Small inorganic NPs, for example, silica NPs, are in clinical trials as positron emission tomography—optical imaging agents for lesion detection and cancer staging.10

Although nanomedicine has made tremendous progress, it is still questionable whether the EPR effect is sufficient to significantly improve the survival of patients with cancer by nanomedicine.11 Indeed, the abnormal tumor microenvironment contains several delivery barriers to limit the transport of NPs deep into tumors (Fig. 1).12 The distribution of NPs from the bloodstream into tumors is impeded by tumor blood flow stasis or collapsed tumor blood vessels.3 The excessive vessel leakiness and blood flow stasis also bring out high interstitial fluid pressure that considerably hinders the extravasation of NPs.14 The NP access deep into tumors is often hindered by the large distance between blood vessels in tumors15,16 and by the dense interstitial matrix, a complex assembly of collagens, glycosaminoglycans, and proteoglycans.17 For example, Doxil and Abraxane are found trapped less than 100 nm away from vessels.18,19 Recent advances in biology also show that many factors in the tumor microenvironment contribute to the tumor escape from immune system surveillance, including the expression of T cell–inhibitory molecules,20 the activation of T cells resulting in anergy,21 and the presence of both CD4+CD25+ T cells and CD1d-restricted T cells that suppress antitumor immunity.22,23 Such abnormal tumor microenvironments help tumor progress and resist the treatment. Therefore, the nanomedicine has to be designed to overcome the delivery barriers in tumor microenvironment to improve the therapeutic efficacy.24

In this review, we discuss the advances to use nanomedicine to overcome delivery barriers, especially such in the tumor microenvironment. We first discuss recent studies on the physicochemical properties of nanomedicine to develop NP-tumor interaction and to enhance the delivery in tumors (Physicochemical Properties of Nanomedicine). We feature the advances in stimulus-responsive delivery that can drive NP accumulation and penetration in tumor (Stimulus-Responsive Drug Delivery). We highlight some emerging strategies to manipulate tumor microenvironment by nanomedicine, through normalization approaches (Nanomedicine Targeting the Tumor Microenvironment). Nanomaterials for cancer immunotherapy that can change the immunosuppressive tumor microenvironment will also be reviewed (Nanomedicines for Cancer Immunotherapy).

PHYSICOCHEMICAL PROPERTIES OF NANOMEDICINE

To achieve therapeutic efficacy, NPs must first overcome systemic barriers with prolonged circulation time, especially clearance by mononuclear phagocytic system, hepatobiliary system, and urinary system.25 Then NPs extravasate from tumor vessels and penetrate the tumor parenchyma so that even cancer cells situated distal to the tumor vessel can be exposed to the anticancer agent at high-enough concentrations. These nanomedicine delivery processes are largely affected by physicochemical properties of NPs, including size, surface charge and chemistry, and geometry.
Nanoparticle Size

Nanoparticles with sub-100-nm sizes appear to be optimal for the EPR effect.26,27 However, NPs must penetrate up to hundreds of micrometers through stroma to reach cancer cells. Therefore, deep penetration of NPs in tumors is necessary for therapeutic effect.28 Nanoparticle size is a crucial determinant of accumulation and penetration into tumor tissue. Small NPs usually have deeper tumor penetration than large NPs, but there remains discrepancy of intratumoral accumulation trend of different-sized sub-100-nm NPs in many references.26,29-31 It is reported that polymeric micelles ~30 nm showed enhanced tissue penetration and potent antitumor activity in pancreatic tumors, compared with larger NPs.31 In another example, 50-nm silica NPs showed deep tissue penetration and higher accumulation in glioblastoma.32

Nanoparticle Surface Chemistry

In addition to size, the surface chemistry of NP, especially surface charge, affects their tumor penetration. It is reported that cationic NPs extravasate more rapidly in tumors than neutral or anionic particles because of the attractive electrostatic forces between cationic NPs and anionic endothelial glycolectins.33-37 However, neutral NPs display the fastest interstitial transport than charged NPs presumably because of the minimized NPs binding to anionic glycosaminoglycans or positively charged collagens.38-40 Besides tumor penetration, the surface chemistry of NP is well known to influence the NP circulation and biodistribution. Nanoparticles often have prolonged circulation in the blood compared with small-molecule drugs (~5 nm).7,41 Macrophages in the reticuloendothelial system can engulf and clear injected NPs, which can lower the dose of NPs reaching tumors. Moreover, the macrophage uptake of NPs can lead to compromised host defenses (due to the saturation of macrophage uptake capability by NPs),42 release of toxic byproducts (from exposing NPs to highly oxidative environment upon phagocytosis),43 and redistribution of NPs to the liver and spleen that potentially can induce delayed or chronic toxicity.44-46 Coating of NPs with poly(ethylene glycol) (PEG) that mimics a cell’s glycolectin marker of self,47-49 known as “PEGylation,” can suppress protein absorption to NPs and delay the rate of NP uptake and clearance, greatly prolonging circulation time. However, PEGylation cannot eliminate macrophage uptake that is not mediated by serum absorption.50

An intriguing approach to evading phagocytosis of NPs was to graft a synthetic small peptide, which was computationally designed from CD47, a cell surface “marker of self” that impedes macrophage uptake,51 to mimic the CD47-CD172a interaction that inhibits phagocytosis. This peptide prolonged the circulation time of NPs in vivo.52

Nanoparticle Geometry

The aspect ratio of nanomaterials affects their interaction with cells (e.g., uptake),53 in vivo circulation time, and tumor penetration capability.54,55 For example, flexible polymeric cylindrical micelles had much longer circulation times in vivo than their spherical counterparts.56 Interestingly, rigid carbon nanotubes with lengths of 100 to 500 nm and diameters of 1 nm (aspect ratio ~100–500) can be rapidly renally cleared (circulation half time ~6 minutes).57 In addition, it is reported that nonspherical gold NPs, for example, nanorods and nanocages, could penetrate tumors more thoroughly than the similar-sized nanospheres and nanodisks; however, higher intratumoral accumulations were observed for gold nanorods and nanospheres than their nonspherical counterparts.58

STIMULUS-RESPONSIVE DRUG DELIVERY

To enhance the preferential accumulation of NPs or drug release in tumors, there have been increasing efforts to develop stimulus-responsive nanomaterials that utilize endogenous or exogenous stimuli to overcome the limitation of EPR.28,60 Local tumor microenvironmental factors, such as pH (6.7–7.0),61 redox state (hypoxic tumor microenvironment62 and elevated reactive oxygen species generated by tumor cells63), and specific molecules overexpressed in tumor (e.g., matrix metalloproteinases),64 can disrupt NP structure to release loaded drugs; however, only a few utilize endogenous stimuli (e.g., matrix metalloproteinase 2) to enhance NPs’ tumor penetration.28 Exogenous stimuli include electromagnetism, heat, ultrasound wave, and light. Such spatiotemporal control over the activation of materials may drive localization, maximize cargo release at the desired tumor site, improve NPs’ tumor penetration, or change the tumor microenvironment.65,66 Some means of activation such as ultrasonic waves, sophisticated light sources, or strong magnetic fields may not always be practical or cost-effective. Another problem related
to the application of exogenous stimuli is the depth of tissue penetration that can be expected, which has been discussed elsewhere. A major challenge with many stimulus-responsive delivery approaches is to translate relatively complicated designs from the bench to a successful in vivo application. Many triggerable systems have been reviewed elsewhere, here we will highlight the progress in this area.

**Photothermal Therapy**

Local heating of tumors to \( \sim 41^\circ \text{C} \) to \( 43^\circ \text{C} \), known as hyperthermia therapy, has been shown to increase the blood flow to and permeability of tumor vessels. Liposomes have been designed to release drugs when tumors are preheated, and such thermo-sensitive liposomes containing doxorubicin are currently in clinical trials. However, the conventional hyperthermia often takes \( \sim 30 \) to 60 minutes to heat tumors. More rapid heating (within minutes) can be achieved by irradiating metallic NPs that have surface plasmon resonance (e.g., gold NPs and CuS NPs), which efficiently absorb light and convert it to heat. The photothermal properties of gold NPs have been utilized to enhance the accumulation of subsequently administered conventional NPs in tumors (Fig. 2A).

Organic NPs can be also used to enhance the drug delivery utilizing photothermal sensitizer molecules. Nanoliposomes composed of lipid conjugates of the photosensitizer pyropheophorbide (a chlorin analog) can efficiently absorb and transfer light energy into heat for photothermal therapy. The same nanoliposome can also carry doxorubicin for chemotherapy.

**NANOMEDICINE TARGETING THE TUMOR MICROENVIRONMENT**

**Antiangiogenic Therapy for Vasculature Normalization**

Antiangiogenic therapy can "normalize" the tumor vasculature by inducing vessel maturation such that there is increased perfusion and more evenly distributed vasculature within tumors. This normalization has been suggested as a means of modulating tumor microenvironment and perhaps improving NP delivery into tumors (Fig. 1). Recently, it was found that blocking vascular endothelial growth factor receptor 2 in mouse mammary tumors greatly improved the delivery of small NPs (12 nm) but not larger NPs (such as 60 and 125 nm). The explanation for this observation may be that the normalization of the tumor vasculature by anti–vascular endothelial growth factor receptor 2 agent decreased...
the tumor vessel wall pore size, which then only allowed the smaller NPs (<60 nm) to be rapidly transported in tumor tissue.

**Targeting Tumor Extracellular Matrix**

In solid tumors, penetration of macromolecular agents and NPs is affected by tumor stromal barriers such as the extracellular matrix (ECM, e.g., collagen network, and hyaluronic acid). Numerous studies have shown that ECM-degrading enzymes, such as collagenase or hyaluronidase, can improve NP penetration into solid tumors (Fig. 1). However, ECM-degrading agents may increase the incidence of metastasis. The antihypertensive drug, for example, losartan, was recently found to reduce tumor collagen content by blocking angiotensin II receptor 1 and has been successfully used to enhance diffusive transport and efficacy of intravenously administered NPs such as Doxil. However, in a recent multicenter phase II clinical study, combined chemotherapy with gemcitabine and candesartan, a losartan analog, failed to demonstrate prolonged progression-free survival in patients with advanced pancreatic cancer. A safety concern was also raised because hypotension induced by candesartan was observed in some patients. In addition, PEGylated form of recombinant human hyaluronidase (PEGPH20) has recently been introduced into clinical trials (trial IDs NCT00834704, NCT01170897, NCT01959139) with the combination of Abraxane or other chemotherapeutics. It is reported that high doses of PEGPH20 (50 μg/kg) induced severe (grade 3) muscle/joint pain, whereas low doses were generally well tolerated.

**Tumor-Penetrating Peptides for Enhanced Tumor Penetration**

Tumor-penetrating peptides, such as iRGD (a cyclic RGD peptide, CRGDKGPDC) and Lyp-1 (CGNKRTRGC), were identified by phage library screening and were able to enhance drug or NP penetration into tumors. The iRGD peptide is proteolytically degraded into its active form and bound to neuropilin 1, which is expressed in tumor vasculature and tumor cells, and induces endocytic bulk transport through tumor tissue; the detailed pathway for tissue penetration and endocytosis is still being elucidated. Co-administration of such peptides with Abraxane NPs or Doxil liposomes significantly increased their intratumoral accumulation.

**Targeting Tumors Utilizing Tumor Metabolism**

It is known now that tumor cells are highly metabolically active and favor the inefficient anaerobic glycolysis (uptaking glucose). The 18F-labeled fluoro-2-deoxyglucose, a glucose analog, has been widely used in oncology for tumor diagnosis (by positron emission tomography) and staging. Recently, such saccharide metabolism has been utilized to introduce the chemical functional group on the cell surface. For example, aminosugars containing 1 unnatural functional group (e.g., azide) can be taken up by cells and expressed on their surfaces; the introduced functional group can undergo bioorthogonal chemistry to artificially label the cells. Bioorthogonal chemistry refers to a variety of chemical reactions using functional groups that generally do not occur in the host creature and that do not interfere with native biochemical reactions. Such bioorthogonal reactions include azide-alkyne cycloaddition, azide-phosphine Staudinger ligation, and tetracyclic Diels-Alder reactions. A 2-step in vivo tumor-targeting strategy has been developed to enhance intratumoral NP accumulation (Fig. 3). The first step involved treating the tumor with an unnatural glycan containing an azide group, via intratumoral injection. Cancer cells would take up the glycan and express it, with its azide groups, on the cell surface. When NPs containing alkyne groups were administered systemically, they underwent a bioorthogonal reaction with the azides in the tumor, leading to enhanced intratumoral accumulation of NPs. Bioorthogonal tumor-targeting strategies can also be applied to tumor imaging. Tumor cells prelabeled with antibodies modified with cyclooctene were implanted subcutaneously, and perfluorocarbon microbubbles surface-modified with tetracyclic groups were injected systemically and reacted with cyclooctene on the tumor cells, which could now be better imaged by ultrasound in vivo.

Bioorthogonal approaches are intriguing, but the initial step of introducing the unnatural functional group into tumors is often technically challenging.

**NANOMEDICINES FOR CANCER IMMUNOTHERAPY**

Tumor once thought as simply the growth of malignant cells has been proved to be fueled by localized immunosuppression. The idea of a therapeutic cancer vaccine originated with the discovery that patients can harbor CD8+ and CD4+ T cells specific for cancer-testis antigens expressed in their tumors. In addition, clinical studies showed a strong association between prolonged patient survival and the presence of intratumoral CD3+ or CD8+ cytotoxic T cells with an interferon γ gene signature. The understanding of the molecular basis of immune activation, especially the research on T-cell receptors subunits and T-cell receptor costimulatory and coinhibitory molecules, paves the path to current promising cancer immunotherapy with recent success of proof-of-concept clinical trials. One prominent example of

![Image](https://example.com/image.png)

**FIGURE 3.** Schematic illustration of tumor targeting using bioorthogonal reactions. Tumor cells are first fed with unnatural aminosugars containing 1 functional group for the bioorthogonal reaction. That functional group is later expressed on the tumor cell surface and can react via a bioorthogonal reaction with NPs (green sphere) surface-modified with another functional group.
activating the immune system against tumor is the anti–CTLA-4 antibody, ipilimumab, which prevents CTLA-4 from attenuating T-cell activation. The use of ipilimumab achieves a significant increase in survival for patients with metastatic melanoma, which cannot be effectively treated by conventional therapy.\textsuperscript{115} In addition, lambrolizumab and nivolumab, 2 antibodies that target the programmed cell death 1 receptor on activated T cells, remove the brakes imposed by coinhibitory molecules so that cancers can be recognized and destroyed by the immune system.\textsuperscript{116,117}

The use of nanomaterials in cancer immunotherapy can deliver agents to specific organs (e.g., lymph nodes [LNs]) or cells. In particular, NPs have been utilized to target immune cells inside LNs or mucosal tissues to induce immune responses toward tumors. Nanoparticle size directly affects which immune cells the NPs enter.\textsuperscript{118} Upon systemic administration, particles between 500 and 2000 nm are generally processed by antigen-presenting cells (APCs) at the injection site, whereas sub–200-nm NPs can traffic to the LNs where they are captured by LN-resident dendritic cells (DCs).\textsuperscript{119} In another example, after intradermal injection, 25-nm NPs can flow through lymphatic capillaries to the draining LNs, whereas 100-nm NPs cannot be transported to LNs.\textsuperscript{118} Such size-dependent LN targeting has been utilized for both imaging and vaccination. In 1 example, 16-nm iron oxide/zinc oxide NPs carrying carcinoembryonic antigen were injected into the mouse footpad and trafficked to draining LNs. The NPs could be imaged by magnetic resonance imaging, because of the iron oxide, and were also effective as vaccines, showing strong cytotoxic T-lymphocyte responses and significant reduction of tumor growth.\textsuperscript{120} Nanoparticles have been designed to target LNs for vaccination against tumors. The immune-modulator molecule CpG and an adjuvant (ovalbumin) were conjugated onto the surfaces of separate 30-nm polymeric NPs and injected intradermally. Both NP conjugates rapidly drained to the LNs and enhanced the DC cell uptake of both antigen and adjuvant.\textsuperscript{121} This codelivery strategy induced potent effector CD8\textsuperscript{+} T cells and a more efficacious memory recall of cytotoxic T cells upon reinjection of tumor cells, compared with the response with NP-conjugated antigen with free adjuvant.

Nanoparticles can be delivered via pulmonary administration to the numerous APCs in the lung, which can take them up avidly.\textsuperscript{122} A subset of such lung APCs can further transport NPs containing antigens to DCs in draining LNs. In mice vaccinated by pulmonary administration of nanovesicles loaded with antigen and Toll-like receptor agonist that both promote cytotoxic T-cell response,\textsuperscript{123} the antigen was detected in LNs for at least 7 days, whereas pulmonary immunization with soluble vaccines led to rapid antigen clearance. Strong T-cell responses elicited by this pulmonary vaccine nanovesicle enhanced protective immune responses in tumors.

Cell therapy for cancer immunotherapy (e.g., adoptive transfer of T lymphocytes) represents another promising approach.\textsuperscript{124} In this approach, immune cells (e.g., T cells) are harvested and manipulated ex vivo with cytokines to stimulate immune cells, then reintroduced into the body. Cytokines used in such therapy may generate systemic toxicity, but it is necessary to maintain a high level near the administered therapeutic cells in order to maintain cell stimulation over an extended period. A new approach to overcome this problem is to directly tether cytokine-loaded NPs to the surfaces of the therapeutic cells prior to infusion (Fig. 4).\textsuperscript{125} Liposomal NPs containing IL-15Sa and IL-21 were conjugated to thiol groups on the surfaces of T lymphocytes. The NP-tethering strategy greatly enhanced T-cell survival and expansion after infusion and slowed tumor growth (Fig. 4).

Besides using NPs to deliver antigens or immunotherapeutic agents, NPs can trigger adaptive immune response in tumor. Recently, it was reported that the photothermal ablation of breast tumor using single-walled carbon nanotube can induce the immune response at the tumor site, which is not observed on the condition that tumors are removed by surgical resection.\textsuperscript{126} The carbon nanotube-based photothermal therapy induced DC maturation in the tumor-draining LNs with elevated cytokines to trigger immune response against tumors. Such photothermal tumor ablation

![Figure 4](image-url)
combined with anti-CTLA-4 blockade could effectively enhance immune response against reinfected tumor cells, with increased ratio of cytotoxic CD8+ cells to regulatory T cells in reinfected tumor site.

PERSPECTIVE
Cancer nanomedicine is a very rapidly growing field of translational medicine.127 However, overcoming major hurdles in cancer nanomedicine, including NP circulation, biodistribution, tumor targeting, and tumor penetration, requires a better fundamental understanding of the processes involved.11,128 The knowledge of cancer biology and oncology will enhance the rational design of NPs for specific cancers. Effective therapeutics and diagnostics for cancer require delivery to tumors with appropriate temporal resolution to achieve the most favorable pharmacokinetics. Stimulus-responsive drug delivery systems are expected to address this need. Research is needed to develop new strategies to tailor NPs to specific tumor microenvironment, especially to metastatic tumors, which accounts for the majority of cancer deaths.129 The revolution in the understanding of tumor-associated immune system in the past decade may lead to safer and more effective nanomedicine-based immunotherapies. Of note, biocompatibility, toxicity, and numerous formulation issues will remain important for the success of cancer nanomaterials.130

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REFERENCES

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