Nanoparticle targeting of anti-cancer drugs that alter intracellular signaling or influence the tumor microenvironment

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Abstract

Nanoparticle-based therapeutics are poised to become a leading delivery strategy for cancer treatment because they potentially offer higher selectivity, reduced toxicity, longer clearance times, and increased efficacy compared to conventional systemic therapeutic approaches. This article reviews existing nanoparticle technologies and methods that are used to target drugs to treat cancer by altering signal transduction or modulating the tumor microenvironment. We also consider the implications of recent advances in the nanotherapeutics field for the future of cancer therapy.

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1. Introduction

Current cancer chemotherapies often fail to improve patient mortality and morbidity due to severe adverse effects on normal tissues. Targeted nanocarrier systems potentially offer a solution to this problem because they can be designed to deliver cargo preferentially to the tumor cells, sparing healthy tissue from dose-limiting side effects. Because their size and shape can be tailored as needed, nanotherapeutics also enhance bioavailability and plasma solubility.
decrease systemic toxicity, increase patient compliance and improve pharmacokinetics of biologics and small molecules that otherwise have short half-lives in vivo.

The field of nanotherapeutics has been made possible by the development of manufacturing methods that enable synthesis, self-assembly or fabrication of objects with defined size, shape and chemistry on the nanometer scale and that are also biocompatible. These are either composed of multiple molecules or single molecules that are engineered to contain multiple functional cassettes. Examples of biocompatible nanocarriers include those composed of liposomes, polymers, micelles, engineered multi-functional antibodies, and nanoparticles that can be composed of metals (e.g., gold, silver), polymer, quantum dots, dendrimers, fullerenes, ferritin, proteins, DNA, other biological molecules or combinations of these materials (Fig. 1) [1–4]. Nanomaterials also can be precisely designed to achieve desired physical and chemical properties necessary to target delivery of drugs to the dynamic tumor microenvironment with higher therapeutic efficacy and lower toxicity. For example, surface to volume ratio, size, geometry, surface charge, shape and stimuli-responsive properties are examples of design features that can be controlled when formulating nanotherapeutics. Once the nanoparticles are created, they also can be functionalized with additional chemical moieties, such as antibodies or ligands for cell surface receptors, to target to selective sites, prevent clearance from blood, and provide other desired functional properties.

To achieve effective targeting to cancerous tissues, systemically delivered nanoparticles must first extravasate from the bloodstream, pass through the tumor extracellular matrix (ECM), bind to cells, and then cross the surface membrane to enter the target cells and reach appropriate intracellular sites (Fig. 2) [5–7]. Two targeting strategies, one active and the other passive, have been utilized to enhance nanoparticle delivery to tumors. In passive targeting, one capitalizes on the leaky nature of tumor microvessels, which results in enhanced permeability and retention (EPR) responses that facilitate greater accumulation of nanoparticles compared to individual soluble molecules or drugs in the perivascular tumor microenvironment. The EPR effect varies depending on the size and surface properties of the nanocarriers, and hence, nanoparticles must be designed to achieve maximum targeting and therapeutic efficacy. Nanocarriers in the size range of 20–200 nm can easily extravasate through the walls of poorly formed microvessels in the angiogenic tumor niche, and poor lymphatic drainage further enhances their accumulation. Several nanoparticles that utilize this EPR-based passive targeting strategy are currently in human clinical trials [8,9]. Examples include the doxorubicin-liposome carriers Myocet and Doxil [10,11], which have increased half-lives in the blood due to the size of the nanocarrier and in the case of Doxil, chemical coating of the liposome with poly-ethyleneglycol (PEG). Passive targeting polymeric micelles, nanoparticles, and polymer-drug conjugates are also in clinical trials for breast, lung, pancreatic and ovarian cancers [9,11].

The creation of targeting antibodies in late 1970s led to the development of the first active or targeted nanoparticle delivery strategies [12,13]. The concept was simple: by coating nanoparticles with antibodies, they could be selectively directed to sites expressing the target antigen. This active targeted delivery approach was later extended by coating nanoparticles with targeting ligands, lectin-carbohydrates, or antigen molecules that recognize specific sites or endogenous antibodies to facilitate intracellular drug delivery [5,14]; these targeting ligands include peptides, antibodies, aptamers, small molecules and proteins among others [5,14,15]. The density of these ligands on the surface of the nanoparticles also can be fine-tuned to optimize binding and targeting using several physical and chemical immobilization techniques. Examples

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**Fig. 1.** Multi-valent, multi-functional and stimuli-responsive nanocarrier delivery systems for tumor targeting. Examples of nanocarrier delivery vehicles include (left to right) liposomes, polymers, micelles, engineered antibodies and nanoparticles decorated with stimuli-responsive cleavable stealth coatings (blue rectangles), targeting moieties (arrows), and anti-cancer drugs or imaging agents (stars and suns).
include technologies that enable precise control of size, shape, composition and surface modification of nanoparticles [16,17] and a lipid insertion method that is used for targeted functionalization of erythrocyte-cloaked nanoparticles [18]. There are only a few active targeted cancer nanotherapeutics in clinical trials. The two that are farthest along are doxorubicin-containing immunoliposomes that are targeted to cancers using a human monoclonal antibody, and transferrin-targeted oxaliplatin liposomes; however, most of the published results on these nanotechnologies refer to preclinical studies [11,19,20].

The most common anti-cancer nanotherapeutics in development target receptors on the surface membranes of tumor cells to enhance local drug concentrations, facilitate drug entry into the cell, and selectively perturb specific receptor-mediated signaling pathways [5]. However, as our understanding of cancer biology has advanced, there has been increased recognition of the importance of intracellular signaling molecules and particular genes for cancer development, as well as new appreciation for the key role that the tumor microenvironment (e.g., capillary blood vessels, stroma, ECM composition and mechanics, immune cells, etc.) contributes to cancer control. Thus, nanocarriers are also being employed to selectively concentrate drugs at tumor sites based on the tumor microenvironment, as well as to target specific intracellular signaling molecules, and genes. Nanoparticles with multivalency, multi-functionality, and triggered or stimuli-responsive release properties (Fig. 1) are all currently being explored for tumor targeting [6,21–23]. Clearly, there are still many challenges that need to be overcome before we can design fully effective, targeted, nanoscale, drug delivery systems that will overcome the complex interactions between the tumor cell’s intracellular information processing network and the ever-changing tumor microenvironment that govern cancer formation and expansion. Thus, in this review, we focus on various nanoparticle-based drug delivery approaches that are being developed to either target intracellular signaling pathways or the tumor microenvironment, as shown in the schematic in Fig. 3. We also explore the implications of recent developments in this field for the future of targeted cancer therapy.

2. Targeting signaling networks in cancer cells

Cancers are heterogeneous in nature, and their growth and progression depend on reciprocal interactions between tumor cells and their surrounding microenvironment [24–26]. Information processing networks that govern cancer behavior include both chemical and genetic signaling pathways inside individual tumor cells, as well as intercellular signaling networks that involve soluble cues transferred back and forth between tumor cells and surrounding vascular endothelial cells, cancer-associated fibroblasts, and cells of the immune system [24,27,28]. In this section, we examine nanoparticle-based delivery strategies that target drugs to these diverse information handling networks that process soluble cues in the tumor.

2.1. Surface-receptor mediated pathways

Detailed molecular characterization of tumor cell signaling networks has identified changes in growth signaling receptors that characterize specific tumor types. Active targeting of nanoparticles to these signaling components could potentially increase therapeutic effectiveness and reduce collateral damage caused by chemotherapeutic drugs on normal healthy tissues. Molecular recognition of tumor cells may exploit ligand–receptor interactions or antigen–antibody binding to efficiently target the nanoparticle drug delivery vehicle to the tumor. Humanized antibodies are attractive for targeting cell surface receptors due to their high specificity and low immune reactivity [29]. Active targeting via antibody interactions can distinguish cancerous cells from normal tissue and also can target specific subpopulations of cancer cells [30].

Folate is one of the most extensively utilized ligands for targeted cancer nanotherapeutics due to its high affinity binding to the folate receptor ($K_d \approx 10^{-9}$) and because it is frequently overexpressed in diverse tumor types including uterine sarcomas, ovarian carcinomas, osteosarcomas, and non-Hodgkin’s lymphomas [30]. Folate recently was used to target delivery of tamoxifen with albumin and alginate-based nanoparticles [31] and enhanced in vivo targeting of camptothecin to pancreatic xenografts was accomplished through the use of folate-coated mesoporous silica nanoparticles loaded with this drug [32]. Significant reduction in tumor volume and enhanced renal clearance were observed compared with uncoated particles in the camptothecin nanoparticle studies.

Transferrin is an iron-binding plasma glycoprotein that facilitates iron uptake by cells through binding to its membrane receptor, TFR [33], and this receptor is overexpressed by as much as 10-fold in many tumor cell types [34]. Ligand binding of TFR initiates endocytosis, which has been exploited for targeted delivery of transferrin-coated nanoparticles. In vivo examples of this approach include the targeting of transferrin-conjugated paclitaxel-loaded nanoparticles to prostate [34], and delivery of transferrin-coated PLGA-nanoparticles to gliomas [35] in animal studies.

Epidermal growth factor receptor (EGFR) is over-expressed in many cancers and can serve as a target for nanotherapeutics through binding of one of its two natural ligands: epidermal growth factor (EGF) or transforming growth factor-alpha (TGF-α). EGF-conjugation of stearyl gemcitabine nanoparticles (GemC18-NPs) has been shown to result in a
2-fold improvement in its targeting to tumor cells that over-express EGFR [36]. Human epidermal growth factor receptor 2 (HER2) is over-expressed in breast, ovarian, gastric and uterine cancers and it is the target of the monoclonal antibody trastuzumab (marketed as Herceptin). Human serum albumin nanoparticles that were surface-coated with trastuzumab and loaded with the cytostatic drug doxorubicin displayed enhanced cell uptake and biological activity [37]. Such targeted approaches may be key to reducing clinical issues of cardiotoxicity that can be a major source of morbidity in patients receiving doxorubicin. The challenge of doxorubicin resistance also may be overcome, or at least reduced, using nanoparticles that are not recognized by P-glycoprotein, which is one of the main protein pumps that mediate multidrug resistance response.

2.1.1. Transmembrane delivery vehicles

Several transmembrane nanoparticle-based delivery strategies have been reported to permit the delivery of small molecules, proteins, small interfering ribonucleic acids (siRNAs) and imaging agents to intracellular compartments of tumor cells. Small molecule-mediated nanoparticle delivery of small molecules is commonly accomplished by utilizing mechanisms of endosomal escape and cytosolic release of nanoparticles. Delivery of doxorubicin by mesoporous silica nanoparticles coated with polyethylenimine (PEI) [38] and gold nanoparticles coated with doxorubicin conjugated to an acid cleavable PEG linker [39] have both been shown to effectively deliver small molecules into cytosol. More recently, it was shown to be possible to target chemotherapeutic drugs to mitochondria using poly(lactic-co glycolic acid) (PLGA)–PEG nanoparticles blended with triphenylphosphonium, a lipophilic cation known to cross mitochondrial membranes [40,41].

For intracellular protein delivery, silica nanoparticles that target phospho-Akt (protein kinase B) to inhibit its kinase activity have been developed [42]. The nanoparticles enter cells through the endosomal pathway and subsequent escape into the cytosol, causing protein delivery and eventually, cell death. Intracellular protein delivery also has been accomplished by targeting the ribosome inhibiting proteins (RIP) that suppress translation [43]. Several “cell penetrating peptides,” such as the TAT (transactivated-transcription) sequence, have been utilized to mediate transfer of peptides and proteins across cell membranes and directly into the cytosol [44]. These have been used to create nanocarriers that can enter the cytoplasm as well. Examples include TAT-conjugated PLGA nanoparticles to deliver K237 peptides that target the kinase insert domain receptor for brain drug delivery [45], and intracellular delivery of quantum dot-mediated protein delivery and imaging [46].

The physical properties of nanoparticles also have been leveraged to enhance cellular internalization. For example, the specificity of targeting ligand-coated nanoparticles can be augmented by engineering their shape to exhibit distinct 3D geometries. PEG-hydrogel rods with a high aspect ratio of 150 × 450 nm exhibit higher cellular uptake compared with cylindrical particles with 200 × 200 nm dimensions [47]. Uptake and internalization of trastuzumab-coated nanoparticles by breast cancer cells also were found to be higher for rod-shaped particles, followed by disks, and then spheres [48]. Interestingly, rod-shaped particles were also found to provide improved specificity of endothelial targeting in lung and brain compared with other geometries [49]. Thus, the ability to shape nanocarriers provides an alternative way to affect drug delivery, independently of the drug being delivered or their surface coating.

2.2. Targeting intracellular signaling molecules

Intracellular signaling molecules control cell proliferation, differentiation, and survival, as well as myriad metabolic functions in cells. During cancer progression, the signaling networks undergo severe alteration and deregulation. Therapeutic targeting of specific molecules within these complex tumor signaling networks remains a challenge due to poor drug penetration into intracellular compartments. Nanocarriers offer a new way to overcome this challenge because they can be tuned to deliver the cargo by receptor-mediated internalization as well as organelle- or intracellular molecule-specific targeting. Below, we outline some recent developments in this area.
2.2.1. Focused delivery of siRNAs

The recent development of siRNAs, which direct sequence-specific degradation of target mRNA transcripts, has led to the development of an entirely new class of therapeutics for cancer. As the siRNA molecule itself is strongly negatively charged, delivery to cells is typically accomplished by complexing it with a cationic carrier or lipid-based nanoparticle. In the first-in-human trials studies, siRNA was targeted to the M2 subunit of ribonucleotide reductase via cyclodextrin–PEG nanoparticles coated with human transferrin [50]. TIR receptor is expressed at high levels on malignant cells as described above, and thus this enhanced accumulation of the nanoparticles at tumor sites in patients with refractory melanoma.

A new class of nanoparticle materials, termed “lipidoids,” has been developed to facilitate siRNA delivery. Lipidoids are derived from an amine-containing backbone from which aliphatic chains extend; the amines appear to interact electrostatically with the nucleic acid phosphate backbone and facilitate endosomal escape as the compartment becomes acidified. The efficacy of lipidoid nanoparticle-siRNA formulations has been demonstrated in the silencing of tight junction protein claudin-3 in animals models of ovarian cancer [51] as well as Parp1 in Brca1-deficient ovarian tumors [52] and glycolytic enzyme pyruvate kinase in several xenograft models [53]. Injection of lipidoid nanoparticles carrying siHoxA1 through the nipple and directly into the mammary duct prevented the progression of mammary tumors in the C3(1) SV40TAg transgenic mice [54].

Although siRNA delivery by nanoparticles is able to deliver micrograms of drug per kilogram of animal body weight, there remains room for additional improvement. For example, better understanding of the endocytotic pathways of particular siRNA–nanoparticle formulations may improve their efficiency of delivery. After lipid nanoparticles enter the cell, a significant amount of siRNA remains trapped in endocytotic vesicles and does not reach its intracellular target. In parallel, vesicle recycling continues to rapidly clear the siRNA–nanoparticles from the cell before the payload is delivered. One recent study identified Niemann–Pick type C1 (NPC1) as a critical regulator of the major recycling pathways of lipid nanoparticle-formulated siRNAs. In cells lacking NPC1, there was enhanced retention of nanoparticles inside the endosome and lysosomes of the recycling pathway, and the level of gene silencing achieved with RNA interference was 10 to 15 times greater than that produced in normal cells [55]. Strategies to target specific molecular components of endosome production, trafficking and recycling may be incorporated into the design of the next generation of nanocarriers for siRNA delivery. Another challenge is that immune stimulation through α/β interferons, RNA-dependent kinase effects, and Toll-like immunity could limit the safety profiles of siRNAs. However, if this immune activating activity of the siRNAs can be controlled or even exploited for use against the tumor, this side effect could potentially improve treatment efficacy. The current status of nanoparticle-based RNAi therapies in clinical trials for oncology applications is shown in Table 1.

3. Targeting the tumor microenvironment

Over the past few decades, it has become recognized that tumor growth and progression are not solely the result of cumulative gene mutations, but are also significantly influenced by the surrounding tumor microenvironment. In addition to cancer cells, the dynamic tumor microenvironment consists of ECM, stromal cells, endothelial cells, and immune cells that are recruited from surrounding tissues as well as from the bone marrow [25,56]. Interestingly, the chemistry, structure and mechanical properties of the tumor microenvironment all influence the cellular information processing networks that drive phenotypic changes leading to tumor malignancy [24,56–59]. Hence, it is important to consider both cellular and non-cellular components of tumor microenvironment for cancer therapy as their complex interactions play a critical role in cancer progression. In this section we discuss nanoparticle-based delivery systems that have been reported to target various components of the tumor microenvironment.

3.1. Vasculature

In contrast to normal tissues, the vasculature of tumors is marked by a heterogeneous distribution of vessel sizes and shapes, generally larger vessel diameters, higher vascular density, and enhanced permeability. This inherently leaky vasculature promotes preferential accumulation of nanoparticles in the tumor interstitial space, and when coupled with impaired lymphatic drainage of macromolecules, this results in the EPR effect characterized by increased accumulation of nanoparticles that is observed in many solid tumors [60], as described above. The EPR effect has been successfully exploited for passive targeting of drugs to solid tumors in animals that are encapsulated in a variety of high molecular weight carrier systems, including polymeric drug conjugates, liposomes, polymeric NPs and micelles [61–64]. However, below we focus on strategies for active targeting of nanotherapeutics to the tumor vasculature.

3.1.1. Angiogenesis

One of the major angiogenic factors elaborated by tumors – vascular endothelial growth factor (VEGF) – both stimulates new capillary ingrowth and increases the permeability of blood vessels [65]. Targeting the VEGF receptor on endothelial cells is therefore an effective method to prevent cancer growth by blocking tumor angiogenesis. Targeting the VEGF receptor-2 (VEGFR-2) has been investigated as a way to enhance delivery of various nanoparticle systems to tumor vessels, including boronated dendrimers and radioisotope-labeled polymerized liposomes [66]. VEGF targeting also was accomplished in an animal model of hepatic carcinoma using dextran magnetic nanoparticles (DMNs) conjugated to radiolabeled 131I-anti-VEGF monoclonal antibodies [67].

One of the first angiogenic inhibitors TNP-470 demonstrated broad spectrum activity in early trials, including efficacy in metastatic disease in human patients, but then stalled in part due to neurotoxicities. A reformulation of this angiogenesis inhibitor composed of polymeric PEG–PLGA nanoparticles surrounding a TNP-470 core, called Lodamin, was shown to prevent the compound from crossing the blood–brain barrier, eliminating its neurotoxicity, while retaining its potency and broad-spectrum activity [68–70]. The new Lodamin formulation also greatly improved oral availability as the polymeric PEG and PLA protect the inner active compound from degradation by the acidic stomach.

<table>
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<tr>
<th>Drug</th>
<th>Disease</th>
<th>Target</th>
<th>Nanoparticle</th>
<th>Company</th>
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<tr>
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<td>Phase I</td>
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Table 1
Nanocarrier-mediated siRNA delivery systems in clinical trials.
environment. Lodamin is also under investigation as a therapeutic for ophthalmologic diseases of the vasculature including diabetic retinopa-thy and age-related macular degeneration.

Vascular cell adhesion molecule-1 (VCAM-1) is an immunoglobulin-like transmembrane glycoprotein receptor which mediates immune cell adhesion that is expressed at high levels on the surfaces of endothelial cells in tumor blood vessels. Liposome nanoparticles coated with anti-VCAM-1 antibodies selectively target the human tumor vasculature in vivo in xenograft studies [71]. Thus, VCAM-1–targeted nanocarriers could provide a way to both enhance delivery of drugs to growing tumor vessels and alter endothelial function.

Knowledge of the mechanism by which nanoparticles enter vascular endothelial cells also has given rise to modifications that further en-hance targeting. Caveolae-mediated endocytosis is the most prominent transendothelial delivery pathway and has attracted interest because it has the capability to bypass lysosomes. Surface modifications that enhance caveolae targeting, such as coating gold nanoparticles with an antibody to the caveolae–enriched protein aminopeptidase P, have been shown to be effective in concentrating the nanomedicine in lung tumor microvasculature [72].

3.1.2. Hypoxia

Hypoxia is a hallmark of cancer, which results from rapid tumor cell growth that overcomes the rate of vascular ingrowth. Hypoxic regions in tumors experience high interstitial pressure build up due to compres-sion of blood vessels, poor lymphatic drainage and deterioration of structural features of tissues that support diffusion. Hypoxia is also commonly associated with poor circulation, tumor progression, malignancy and multi-drug resistance and hence, the delivery of drugs to hypoxic microenvironments remains a significant clinical challenge in cancer therapy [73–75].

Several nanotherapeutic strategies have been used to develop thera-nostics (combined therapeutics and diagnostics) for hypoxi-environments within tumors. For example, a layer-by-layer assembled polyelectrolyte nanoparticle has been shown to selective target to hypoxic regions of tumors [76]. Targeting is based on the low pH in the hypoxic microenvironment, which triggers shedding of the outer layer of the nanoparticle, revealing a charged nanoparticle surface that is subse-quently taken up by cells. Hypoxia–activated, near-infrared (NIR) light–triggered chitosan nanoparticles also have been developed to deliver drugs to cancer cells [77]. This smart nanosystem consists of a hypoxic sensor, a photactivatable trigger, and a caged drug; as it is highly specific to the hypoxic tumor microenvironment, its selectivity is greatly increased. Polysytrene nanoparticles doped with an oxygen-sensitive NIR–emissive palladium meso-tetraphenylporphyrin that se lectively activate and emit optical signals when they experience hypoxic conditions also have been developed [78]. In vitro, the nanoparticles were easily phagocytosed by murine alveolar macrophages and exhibited sensitivity to low oxygen concentrations resulting from induction of hypoxia inducible factor–1α (HIF–1α). Experiments performed in vivo with tumor-bearing mice confirmed that these nanoparticles can be used as imaging probes for tumor hypoxia; however, the same approach potentially could be leveraged to develop therapeutics.

3.2. Immune response

Renewed recognition of the critical role of the immune system in cancer has driven the development of immunotherapies that target existing tumors and enhance long-term immune surveillance. Nanoparticle carriers improve specificity of immune-cell targeting, permit simultaneous delivery of antigens and immune agents, and enhance sustained release.

Human interleukin 12 (IL-12) induces a potent anti-tumor immune response, but systemic administration of IL-12 in early clinical trials resulted in serious dose-dependent toxicities, including death. In xenograft models, intratumoral injection of IL-12 loaded liposomes resulted in an enhanced memory T cell response [79]. Importantly, the drug did not enter the peripheral blood circulation, suggesting that this could help to improve its safety profile [79].

Biodegradable nanoparticles are emerging as tools for targeting tumor antigens to dendritic cells, which is a critical step in eliciting cyto-toxic T cell activation for antitumor vaccine efficacy [80]. Several studies have demonstrated the successful use of mannose–grafted nanocarriers and an increase in the uptake of these carriers by dendritic cells. For example, PLGA nanoparticles coated with mannans, a natural polysaccharide isolated from the cell wall of Saccharomyces cerevisiae, have been shown to have a stronger binding affinity and produce increased CD4 + and CD8 + T cell responses compared to unmodified nanoparticle surfaces [81]. Other particulate vaccines have been successfully developed to sp ecifically target the mannose receptor, such as mannose–PEG3–NH2-decorated PLGA mannose [82], mannosylated-gelatin nanoparticles [83] and liposomes containing mannosyl lipid derivatives [84]. Targeting antigens to C-type lectin receptors also has been shown to result in enhanced humoral and cellular responses which are sufficient to induce immunity against melanomas tumors in vivo [85]. Moreover, similar targeting can be accomplished using anti-lectin antibodies instead of lectin ligands [86].

Another attractive property of nanoparticle carriers is that they offer the potential to deliver both antigen and adjuvant simultaneously to the same cell, thus improving the sustained immune response. Several Toll-like receptor (TLR) ligands have been tested as adjuvants of cancer vac-cines in clinical trials for various cancers, including melanoma, lympho-ma, glioblastoma, breast, prostate, ovarian and lung. As some TLR ligands have severe systemic side effects, nanoparticle delivery to the desired site of action can greatly improve their safety profile and permit use of lower doses. In addition, nanocarriers can improve intracellular uptake, thus expanding the range of targeting options to intracellular receptor family members such as TLR3, 7, 8 and 9 [87].

Another approach uses programmable macroporous 3D PLGA scaf-folds containing nanoparticles to induce endogenous dendritic cells to recognize tumor antigens placed within the scaffold, and to mount a systemic anti-cancer response in vivo [88]. The scaffolds contain tumor antigens from biopsy samples along with PLGA microspheres that release the chemokine GM-CSF to recruit the dendritic cells, and polyethyleneimine (PEI)–CpG cationic nanoparticles containing the immune adjuvant CpG to stimulate dendritic cell maturation. When im-planted in a mice tumor melanoma model, this nanoparticle-enabled vaccine resulted in survival of 90% of tumor–bearing animals [88] and intracranial regression of glioma has been demonstrated in rats [89]. These programmable scaffolds comprising micro and nanoparticles show potential in future cancer immune therapy, and human Phase I clinical trials were recently initiated with this technology.

One new approach to stimulating the host immune response is using light–activated nanoparticles: biodegradable polymer and zinc phthalo-cyanine (ZnPc) photosensitizer nanoparticles target the mitochondria of cancer cells and subsequent light activation elicits the production of tumor antigen, triggering dendritic cell activation [90]. These ex vivo studies suggest that secretd compounds from mitochondria–targeted and light–activated cancer cells also might be useful as cancer vaccines.

3.3. Extracellular matrix

Tumors have a dense ECM consisting of highly interconnected cross-linked collagen fibers and associated large glycoproteins and proteogly-cans. Changes in the structure and mechanical properties of the ECM can alter cellular mechanical signaling pathways and thereby influence subsequent tumor progression and metastasis [57,91,92]. For example, during tumor progression, overexpression of the ECM–modifying en-zyme lysyl oxidase (LOX) results increased collagen cross-linking, thereby increasing ECM rigidity and tissue tension [93,94]. Moreover, experimental inhibition of LOX enzyme activity suppresses tumor growth and prevents cancer progression, and this is mediated by decreasing
collagen cross-linking and restoring more normal-like tissue compliance [91,95]. Importantly, when blocking LOX antibodies were delivered on biodegradable PLGA-based polymer nanoparticles (~200 nm diameter) created using self-assembly, they produced more effective tumor inhibition in animals using lower doses, and they had a higher therapeutic index (Fig. 4) [96]. Compared to systemic injection of free LOX antibody, this LOX antibody-coated nanotherapeutic exhibited significant tumor inhibition with only ~1/30th dose normally used for systemic administration.

Proteoglycans are also a major contributor to ECM mechanics and in particular, studies have focused on the roles of hyaluronic acid (HA) and the proteoglycan versican in tumor ECM mechanics. Interaction between versican with HA and its cell surface receptor, CD44, serves to modulate the ECM mechanical environment, thereby altering cancer cell proliferation and migration [97,98]. Several studies have utilized HA-conjugated nanocarriers to target CD44 over-expressing cancer cells and to deliver anti-cancer drug to the tumor site. For example, intelligent core-corona HA–PEG–PCL nanoparticles have been used to deliver doxorubicin in Ehrlich ascites tumor-bearing mice, and HA-decorated PLGA nanoparticles enhance doxorubicin uptake by cultured human breast adenocarcinoma cells [99,100]. Efficient CD44 targeting also has been accomplished using HA-modified magnetic nanocrystals for MRI imaging of breast cancer cells [101]. Importantly, HA is also a viable target for cancer delivery (independently of CD44) because it is often over-expressed during tumor progression. Hyaluronidase liposomes disrupt the HA matrix when injected intravenously in a human osteosarcoma solid tumor model [102] and silica nanoparticles coated with hyaluronidase enzyme have been used to deliver adjuvant drugs to treat skin cancer [103].

Apart from activation of chemical signaling, the tumor stroma physically limits the penetration of nanotherapeutics and molecular drugs into the tumor. For this reason, co-delivery of proteases, such as collagenase, gelatinase and hyaluronidase, significantly aids in breakdown of the dense ECM barrier and thereby, increases transport of nanoparticles into the tumor and enhances targeted delivery [1,104,105]. Other enzymes (e.g., transglutaminase) and proteins (e.g., the proteoglycans lumican, decorin and biglycan) also may play a role in modifying tumor ECM properties and influencing cancer development [106–111].

**Fig. 4.** A) Schematic of the process by which lysyl oxidase-antibody (LOX-ab) coated PLGA nanoparticles are formed by solvent displacement, aqueous self-assembly and surface conjugation. B) Size characterization of the LOX-ab nanoparticles using transmission electron microscopy (TEM) (left) and dynamic light scattering (DLS) (right) confirming that their size is ~200 nm in diameter and that they are spherical in form. C) The therapeutic potential of these nanoparticles was demonstrated by injecting the LOX-ab coated nanoparticles intravenously in a mammary tumor xenograft model; this resulted in significant inhibition of tumor growth when analyzed over time (left) or by visual inspection at the end of the experiment after removal of the tumors (right) (adapted with permission from Kanapathipillai et al. [96]).
example, macromolecular drugs (e.g., FITC-dextran, FITC-albumin) penetrate more deeply into tumor tissue after degradation of collagen and decorin in the ECM [112]. Thus, nanoparticle-based strategies to modulate these ECM enzymes and protein functions, will likely improve cancer targeting specificity, drug diffusion and accumulation, as well as drug retention at the tumor site.

The ECM is a dynamic structure as it is maintained through ongoing coupling between synthetic and degradative mechanisms; however, ECM remodeling becomes deregulated during tumor progression [57] and this is mediated by elevated expression and activity of various proteolytic enzymes, including matrix metalloproteinases (MMPs) and urokinase-based plasminogen activator systems [113,114]. Interestingly, several nanoparticle-based theranostics have been designed to both monitor MMP expression and inhibit their activity in the tumor microenvironment. For example, liposomes modified with the GPLPLR peptide sequence to target MT1-MMP showed higher binding efficiency and higher therapeutic efficiency in tumors than uncoated liposomes [115]. Nanoparticle-based prodrug strategies also have been developed by taking advantage of MMP-sensitive activation mechanisms which are selectively triggered in the tumor microenvironment. An albumin-binding doxorubicin prodrug that is sensitive to MMP2 has been shown to be effective in malignant melanoma [116]. Gold nanoparticles conjugated to MMP-sensitive peptides also have been used in tumor imaging and drug delivery [117,118]. Recently, MMP-2-sensitive denuding of gold nanoparticles in human breast adenocarcinoma cells has been shown to improve their uptake and hence, enhance targeted delivery to tumor cells [119]. Tumor-triggered release of doxorubicin from MMP-sensitive gold nanoparticles also has been demonstrated in a SCC-7 subcutaneous tumor model [120].

The serine protease inhibitor, urokinase (uPA), plays a major role in tumor cell invasion and metastasis and uPA-targeting nanoparticles have been developed for tumor targeting and imaging. Magnetic nanoparticles coated with uPA receptor (uPAR)-targeting peptides have higher rates of uptake in uPAR positive cancer cells compared with uncoated particles [121], and uPA-targeting iron oxide nanoparticles have been used for in vivo imaging of breast cancer [122]. These nanoparticles were coated with a recombinant peptide of the amino terminal fragment of uPA, which targets the uPAR that is overly expressed in the breast cancer tissue microenvironment.

Nanoparticles or small molecule drug carriers also have been developed to target tumor support structures. The cyclic decapetide, CCLIQKNEC, which is known to bind fibronectin–fibrin complexes in the tumor ECM, has been conjugated to gadolinium–containing dendrimers for targeting breast tumor xenografts [123]. The tumor stroma also contains an abnormal meshwork of clotted plasma proteins, such as fibrin. The plasma clot-binding peptide, CREKA, which was selected using phage display [124] has been used for targeted delivery to the fibrin mesh in tumor stroma [125]. Nanoparticles and liposomes were conjugated to the CREKA peptide and shown to both target to the tumor stroma and also to induce self-triggered clotting.

3.4. Multi-drug resistance

Nanoparticles also have the potential to overcome multi-drug resistance (MDR) that can severely restrict the effectiveness of conventional chemotherapeutic drugs in the tumor microenvironment [126]. Due to non-specific and ligand receptor-mediated targeted uptake, nanoparticles are able to bypass efflux by ABC transporter proteins and accumulate intracellularly; this is in contrast to free drugs which can be highly susceptible to ABC transporter P-glycoprotein (P-gp) capture and efflux [127,128]. For instance, P-gp efflux and multidrug resistance could be overcome in cultured P388 leukemia cells using doxorubicin-loaded poly(alkyl-cyanoacrylate) nanoparticles [129,130]. In murine cancer models, these nanoparticles circulated longer and displayed increased accumulation in tumor tissue when coated with PEG-modified drug.

Another strategy to overcome MDR is to exploit the cell-surface receptor binding strategies of nanoparticles. Studies using transferrin-conjugated nanoparticles or liposomes-containing paclitaxel and oxaliplatin, as well as pH-stimuli-responsive polymeric micelles containing doxorubicin, have all shown less development of drug-resistance in tumor mouse models compared to free drug [20,34,131]. Other recent studies on MDR-modifying nanoparticles with increased therapeutic efficacy include doxorubicin-loaded lipid nanoparticles in human breast and ovarian cancer cells [132], anti-P-glycoprotein-conjugated PEG–PLGA, and poloxamer-modified PLGA nanoparticles for the targeted delivery drugs to cervical carcinoma cells over-expressing P-gp [133].

4. Challenges for the future

While there have been many great advances in the area of nanocarrier-based drug delivery over the past few decades, many challenges remain for the design of effective nanotherapeutics for clinical cancer therapy. Nanomaterials that target specifically to the cancer cell or the tumor microenvironment, but do not exhibit long-term toxicity and can be effectively cleared from the circulation and removed from the body, without compromising circulation time or bioavailability must be developed [134]. A deeper understanding of the complexities involved in cancer cell physiology and the tumor microenvironment, as well as drug and particle pharmacokinetics are key for the development of successful new cancer therapeutics. It will be crucial to develop smart nanomaterials that improve stability, extend circulation time, enhance targeting and improve retention at the tumor site, while minimizing short- and long-term systemic toxicities. Emerging nanoparticle technologies for the delivery of siRNA, anti-metastatic agents, and multi-resistant drugs [8,135,136] show promise, and demonstrate the ability to overcome many of the roadblocks that have limited the effectiveness of conventional drug delivery approaches. More recent identification of CD47 as a “marker of self” which helps to evade phagocytosis uptake of nanoparticles by macrophages, and is also overexpressed in several solid tumors [137,138], raises the possibility of further increasing the effectiveness of nanocarrier drug delivery systems. In fact, nanobead circulation times and therapeutic efficacy were shown to be significantly enhanced in lung carcinoma-bearing mice when the nanoparticles were decorated with CD47 or anti-CD47 antibodies [138].

Another emerging technology in nanotherapeutics is the development of DNA-based nanostructures that form through programmable self-assembly [139,140]. The ability to create virtually any 3D shape using this approach provides a potentially powerful new way to tailor nanoparticle design to optimize its function. For example, self-programmable DNA nanorobots that are capable of selectively delivering anti-cancer payloads (ligands for apoptosis-inducing receptors) only when they bind to specific types of cancer cells (Fig. 5) were recently developed [141]. Exploration of the value of this approach for treatment of human cancer will require demonstration of biocompatibility and stability of these materials when injected in vivo. However, the range of design control and the programmability of these nanoscale robots are huge, and hence, this area will be interesting to watch over the next decade.

Further, in vitro tumor engineering and tumor organ on a chip technologies could serve in optimal design and screening of nanoparticles prior to preclinical studies. For example, the potential of using tumor spheroids for drug screening and delivery to pathological tissues has recently been reported [142]. Spheroids resemble the 3D architecture of tumor tissues, mimicking cell–cell interactions and diffusion limitations of mass and fluid transport, and therefore represent a more suitable platform for testing drugs and nanotherapeutics than conventional planar culture substrates [143,144]. A microfluidic tumor culture device or “tumor-on-a-chip” model, also has been developed to study nanoparticle transport in tissues [144]. Tumor-like spheroids were incorporated into the microfluidic channels and accumulation of nanoparticles was
measured in real-time at physiological blood flow rates. In a further advancement of this organ-on-a-chip approach, the in vivo microenvironment of a breathing human lung alveolar-capillary interface was recreated in a microfluidic chip [145], and this platform was shown to be effective at detecting both physiological nanoparticle absorption and relevant toxicities in vitro [146]. Hence, these organ-on-chip devices might be used in the future as a replacement for animal studies for rapid testing nanotherapeutic efficacy as well as toxicity. In this manner, emerging tumor engineering technologies may synergize with new advances in nanobiotechnology to accelerate the development of more effective nanocarriers while reducing both the time and cost of investigations required to meet these goals.

However, to develop truly effective cancer nanotherapeutics, we have to focus beyond the design of the nanoparticle itself and instead, learn more about the process of cancer formation and progression as well as how we might more selectively interfere with, or prevent, these critical processes. For example, as it is becoming increasingly clear that cancer is not merely a disease of uncontrolled cell growth, and instead that it is a disease of normal tissue development [26], then we might think to explore ways to harness knowledge about development control to create new cancer therapeutics. In this context, it is interesting that developmental biology studies have shown that when ECM isolated from embryonic tissue is combined with adult epithelial cancers, it is capable of normalizing the cancer by suppressing growth and stimulating tissue-specific differentiation [147,148]. Thus, development of biomimetic nanomaterials that mimic the inductive cues found within embryonic ECM could lead to the development of novel nanotherapeutics that might normalize the cancer tissue microenvironment rather than simply kill all growing cancer cells [26]. Interestingly, molecules within embryonic ECM that are responsible for this type of cancer-normalizing activity (e.g., biglycan) are beginning to be identified [111]. Given the key role of ECM mechanics in cancer development, it also might be possible to design nanotherapeutics that prevent tumor growth or normalize the cancer phenotype by selectively modulating the mechanical or structural properties of the tumor microenvironment [26]. This could be facilitated by development of smart nanotechnologies that have programmable mechanical properties, or that can be triggered to alter their physical properties by microenvironmental cues, as is possible, for example, with self-assembling DNA nanostructures [149].

In conclusion, it is clear that simultaneously advancements in genomic, proteomic and nanotechnology fields have enabled many types of new nanotherapeutics to be developed that offer the potential to transform cancer therapy. The newest wave of nanomaterials are multifunctional, programmable, and even self-assembling; they also offer ways to provide longer circulation times, higher selectivity, and more effective targeting to tumor sites. The recent clinical approval of the liposomal-doxorubicin nanoparticles, Doxil and Abraxane [1,11], has validated the value of nanotechnology for cancer treatment. However, the future is even brighter, and the continuing exciting advances in development nanoparticle-based therapeutics will hopefully attract many new investigators in various disciplines into this exciting interdisciplinary field.

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