AAPS PharmSciTech, Vol. 15, No. 3, June 2014 (© 2014)
DOI: 10.1208/s12249-014-0089-8

Review Article

Theme: Translational Application of Nano Delivery Systems: Emerging Cancer Therapy
Guest Editors: Mahavir B. Chougule and Chalet Tan

Nanodrug Delivery Systems: A Promising Technology for Detection, Diagnosis, and Treatment of Cancer

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Received 5 November 2013; accepted 17 January 2014; published online 19 February 2014

Abstract. Nanotechnology has enabled the development of novel therapeutic and diagnostic strategies, such as advances in targeted drug delivery systems, versatile molecular imaging modalities, stimulus responsive components for fabrication, and potential theranostic agents in cancer therapy. Nanoparticle modifications such as conjugation with polyethylene glycol have been used to increase the duration of nanoparticles in blood circulation and reduce renal clearance rates. Such modifications to nanoparticle fabrication are the initial steps toward clinical translation of nanoparticles. Additionally, the development of targeted drug delivery systems has substantially contributed to the therapeutic efficacy of anti-cancer drugs and cancer gene therapies compared with nontargeted conventional delivery systems. Although multifunctional nanoparticles offer numerous advantages, their complex nature imparts challenges in reproducibility and concerns of toxicity. A thorough understanding of the biological behavior of nanoparticle systems is strongly warranted prior to testing such systems in a clinical setting. Translation of novel nanodrug delivery systems from the bench to the bedside will require a collective approach. The present review focuses on recent research efforts citing relevant examples of advanced nanodrug delivery and imaging systems developed for cancer therapy. Additionally, this review highlights the newest technologies such as microfluidics and biomimetics that can aid in the development and speedy translation of nanodrug delivery systems to the clinic.

KEY WORDS: cancer therapy; liposome; nanodrug delivery systems; nanomedicine; polymer nanoparticles.

INTRODUCTION

In the last decade, nanotechnology has tremendously impacted the field of medicine through advances in drug delivery (1). Nanotechnology-based drug delivery aims to target the drug payload to the right place, at the right time, at the right (optimal) dose (2). In general nanodrug delivery systems are submicron-sized particles with one or more therapeutic agents that are dispersed, adsorbed, or covalently bound in encapsulated vesicles, capsules, or polymer matrices (3). Nanodrug delivery systems enhance the bioavailability of each drug reducing deleterious side effects caused by related toxicities (4). In medicine, many of the nanotechnology breakthroughs have occurred in cancer therapy, including drug delivery systems based on polymer nanoparticles or liposomes and image contrast agents in nanoscale dimensions to aid diagnostic imaging or image-guided therapy (5). Liposomal doxorubicin (Doxil®) and albumin-bound paclitaxel (Abraxane®) are among the first generation of nanomedicines bringing therapeutic benefits to cancer patients. These nanoscaled formulations are nontargeted drug delivery systems designed to improve the pharmacokinetics and bioavailability of encapsulated therapeutics.

Realizing the potential of nanoparticles in medicine, researchers worldwide have put tremendous efforts into the development of nanoparticle-based drug carriers resulting in an exponential accumulation of novel nanoparticle systems and related research data. Importantly, many nanotechnology-based systems are rapidly advancing towards preclinical and clinical trials for cancer diagnosis and therapy (6). However, before propelling a new nanoparticle formulation from the bench to the bedside several challenges must first be addressed (7). Ideally, while in circulation, nanoparticle formulations should be stable and inert towards blood components. The carrier should protect the drug(s) from systemic degradation while promoting controlled release properties at

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the tumor site (8). Additionally, the translation of promising nanodrug delivery systems for cancer therapy will be accelerated with an improved understanding of various kinds of targeting moieties and biomarkers. The present review focuses on the recent advancements and translational approaches in nanoparticle-based drug delivery technology in cancer therapy.

NANOPARTICLE PLATFORMS: EMERGING TRANSLATIONAL NANODRUG DELIVERY SYSTEMS

At present liposome-based nanodrug delivery systems have cornered the market for cancer treatment (9). Liposomes are vesicles composed of a lipid bilayer surrounding an inner aqueous compartment. Water-soluble anti-cancer agents are entrapped in the aqueous compartment of liposomes, whereas hydrophobic drugs are incorporated into the lipid bilayer. For example, the first Food and Drug Administration (FDA) approved liposomal doxorubicin formulation (Doxil®) has a lipid bilayer composed of polyethylene glycol (PEG) modified-1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt (DSPE), fully hydrogenated soy phosphatidylcholine, and cholesterol (10). Doxorubicin hydrochloride was then entrapped in the inner compartment of the liposome. Doxil® has been shown to be successful in the treatment of ovarian cancer, AIDS-related Kaposi's sarcoma, and multiple myeloma (10–12). Myocet®, another FDA-approved liposomal doxorubicin formulation, is composed of doxorubicin citrate encapsulated in egg phosphatidylcholine and cholesterol in a 1:1 ratio (13). In 2012, the FDA granted accelerated approval for the treatment of acute lymphoblastic leukemia with the liposomal formulation of vincristine sulfate (Marqibo®; 14). Considering their compositional differences, all of these liposomal formulations differ in their pharmacokinetics and toxicity profiles. Regardless, the drug therapeutic indices were remarkably enhanced through liposomal drug delivery. Recently, platinum drugs have also been reformulated with liposomes because of their otherwise poor oral bioavailability, intense toxicity, and drug resistance. Importantly, multiple liposomal cisplatin formulations (LiPlaCis®, Aroplatin®, SPI-77, Lipoplatin®, and Lipoxal®) have entered various clinical trial phases (14). Table I presents a summary of a few clinically relevant liposomal formulations, which are currently under investigation in clinical trials or FDA approved for cancer therapy.

Advances in nanomedicine have provided new opportunities to combine clinically established liposomal formulations with nanoparticle-based diagnostic imaging. For example, in a recent report, nanoparticles, such as fluorescent quantum dots (Qdots) or superparamagnetic iron oxide nanoparticles, are incorporated in liposomes with doxorubicin for simultaneous delivery of imaging and therapeutic agents, respectively (15,16). Researchers have also attempted to coat lipid bilayer over Qdot-labeled non-enveloped adenoviral vectors for safe and efficient delivery of tumor suppressor genes and imaging agents (15,16). This typical strategy of lipid modification enhanced the tumor accumulation efficiency of the viral vectors and improved the biocompatibility of the Qdots. However, it adversely altered the viral tropism to the liver.

PEGylated liposomes confer long circulating property and stability to encapsulated Qdots leading to reduced liver uptake (17). In a recent study, Qdots were covalently linked to a liposomal lipid layer as a novel formulation strategy (18). Lipid conjugation not only improved the charge characteristics of Qdots but also reduced the toxicity previously caused by leaching of loosely bound Qdots into the circulation (18). Another study demonstrated that the toxicity of Qdots is minimized when encapsulated in liposomes (19). The liposomes carrying Qdots were folate conjugated and showed efficient targeting with high fluorescence yield compared with free Qdots. Although such hybrid nanoconstructs have not yet entered clinical trials, attempting such novel strategies has been a driving force for quick translation of nanoparticle-based drug delivery systems.

Liposomes or lipoplexes are also well known for gene therapy applications and form a major class of lipid-based gene delivery vehicles (20,21). Lipid reagents such as 1,2-bis[oleoyloxy]-3-[trimethylammonio]propane (DOTAP), dioctadecylamido-glycylspermine, *N*-[1-(2,3-dioleyloxy)propyl]-*N*,*N*,*N*-trimethylammonium chloride, and 3β[*N*-(*N*′,*N*′-dimethylaminoethane)-carbamoyl] cholesterol are widely used for gene delivery applications (22). Given their high density of positive charges, these lipids are generally mixed with adjuvant lipids such as cholesterol to reduce the energy required to separate the ionically linked DNA and cationic lipids. This strategy is known to increase transfection efficiency compared with liposomes composed of a single cationic lipid (23).

Previous studies from our research group have demonstrated that delivery of therapeutic genes by lipid nanoparticle systems is a promising strategy for cancer therapy. We have identified the potential of liposomal vectors to deliver *in vivo* tumor suppressor gene *FUS1* to lung tumor-bearing mouse models (24). Restoration of *FUS1* using DOTAP/Chol liposomal delivery system in lung cancer resulted in tumor growth suppression, inhibition of lung metastasis, and prolonged survival of experimental animals (24). These preclinical studies were followed by a phase I clinical trial using systemically administered DOTAP/Chol *TUSC2/FUS1* formulation for patients with primary and metastatic lung cancer. Excitingly, we observed promising outcomes, including efficient uptake of the gene therapeutic by cancer cells, followed by gene product expression, and specific alterations in *TUSC2* pathway begetting anti-tumor effects (25).

Recently, a new gene therapy approach has utilized ultrasound-sensitive liposomes, commonly referred to as bubble liposomes, to transfer gene therapeutics. Bubble liposomes contain ultrasound imaging gas that when combined with ultrasound energy collapse to efficiently deliver the gene therapeutic. Bubble liposomes are fabricated from liposomes prepared by reverse-phase evaporation and perfluropropane gas. Typically, the liposomal suspension is pressurized with excess of perfluropropane gas in a closed vial. Later, the vial is placed in a bath sonicator to form bubble liposomes carrying perfluropropane gas. This technology has recently been used in a mouse tumor model to deliver *interleukin 12* (IL-12) where a dramatic suppression of tumor growth was observed (26). Another study has reported the application of bubble liposomes modified with AG73 peptide in ultrasound imaging of tumor neovasculature (27). Although bubble liposome in combination with ultrasound holds promise in cancer therapy, a more thorough understanding of the physicochemical

Table I. Clinically relevant liposomal formulations

Formulation	Lipid composition	Encapsulated drug	Indication	Clinical status
Antibacterial				
Doxil®	mPEG-DSPE, HSPC, and cholesterol	Doxorubicin hydrochloride	Ovarian, Kaposi's sarcoma, and Multiple myeloma	Approved
Myocet®	Phosphatidylcholine and cholesterol	Doxorubicin citrate	Ovarian, Breast, Kaposi's sarcoma, and Myeloma	Approved
Alkaloid				
Marqibo®	Sphingomyelin and cholesterol	Vincristine sulfate	ALL	Approved
Alkylating				
LiPlaCis	N/A	Cisplatin	Solid tumors	Phase I
Aroplatin®	DMPC/ DMPG	NDDP	Colorectal, and Malignant pleural mesothelioma	Phase II
SPI-77	HSPC, mPEG-DSPE, and cholesterol	Cisplatin	Ovarian, NSCLC, and Head and neck	Phase II
Lipoplatin®	DPPG, SPC-3. cholesterol, and mPEG-DSPE	Cisplatin	NSCLC, Pancreatic, Head and neck, Mesothelioma, Breast, and Gastric	Phases II and III
Lipoxal	N/A	Oxaliplatin	Advanced gastrointestinal tract	Phase I
Gene therapy				
DOTAP/ Chol	DOTAP and cholesterol	*TUSC2/ FUS1*	Primary and Metastatic lung cancer	Phase I

Abbreviations. *ALL* acute lymphoblastic leukemia, *DMPC* 1,2-dimyristoyl-sn-glycero-3-phosphocholine, *DMPG* 1,2-dimyristoyl-sn-glycero-3-phospho-(1′-rac-glycerol) (sodium salt), *DOTAP* 1,2-*bis*[oleoyloxy]-3-[trimethylammonio]propane, *DPPG* dipalmitoyl and phosphtidyl glycerol, *HSPC* hydrogenated soy phosphatidylcholine, *mPEG-DSPE* methoxy-polyethylene glycol-distearoylphosphatidyl-ethanolamine, *N/A* not available, *NDDP bis*-neodecanoatediaminocyclohexane platinum, *NSCLC* non-small cell lung carcinoma, *SPC-3* soy phosphaytidyl choline-3

properties, such as variations in volume occupied by the gaseous material and structure of the bubble liposomes is required.

Another new approach for cancer therapy includes the development of pH-sensitive liposomes (28). pH-sensitive liposomes are stable in physiological pH (pH 7.4), but once internalized by the cell and trafficked to the endosome, which has an acidic pH (early endosomal pH 6.0–6.5), the liposomal structure is destabilized leading to the cytoplasmic release of the liposomal contents (29). Additionally, the extracellular environment of solid tumors is acidic thus acting as a natural signal for triggered liposomal drug release (30). pH-sensitive liposomes are fabricated by different methods. For example, Mo *et al.* (31) used zwitter ionic oligopeptide lipids for the construction of pH-sensitive liposomes. Incorporation of amino acid-based lipids, such as 1,5-dioctadecyl-l-glutamyl 2-histidyl-hexahydrobenzoic acid and 1,5-distearyl *N*-(*N*-α-(4-mPEG2000) butanedione)-histidyl-l-glutamate (18) into the liposomal structure provided multistage pH responses to the tumor microenvironment followed by the endosomal/lysosomal compartment. These pH-sensitive liposomes increased the anti-tumor efficacy of coumarin-6 in a mouse renal cancer xenograft model (31). In a different study, Banerjee *et al.* (32) demonstrated that poly-styrene-co-maleic acid undergoes conformational change at acidic pH resulting in destabilization of the liposomal structure and release of the encapsulated drugs. This polymer–liposome complex was cytocompatible and delivered higher concentrations of the anti-cancer agent 5-fluorouracil (5-FU) compared with conventional liposomes in colon cancer cells. Thus, the development of pH-sensitive liposomes might be a significant step toward the development of an additional clinically relevant nanodrug delivery system. Similarly, the recent development of the temperature-sensitive liposome may hold great potential for applications in cancer therapy. For example, ThermoDox® is a temperature-sensitive nanodrug delivery system that is under evaluation for the treatment of recurrent breast cancer in a phase III clinical trial. ThermoDox® is a liposomal doxorubicin formulation that releases the anti-cancer agent upon exposure to a sublethal temperature of 39.5°C. This strategy allows the localized delivery of a high doxorubicin concentration into the tumor mileu (33).

Polymer nanoparticles also form an important platform for advanced nanodrug delivery in cancer (34). Polymers can be divided into two subtypes: synthetic polymers, such as polylactic acid, polylactic-co-glycolic acid (PLGA), polyhydroxyalkanoate, poly(methyl methacrylate) and natural polymers, which includes gelatin, albumin, chitosan, and alginate. Physicochemical properties of the polymer determine the fabrication process employed to form matrix-based nanoparticles or nanocapsules (35,36). Therapeutics are then entrapped, adsorbed, or encapsulated into these nanoparticles or nanocapsules. Additionally, polymer nanoparticles have the advantage of surface functional groups that can be further explored for modification with targeting ligands (37). Abraxane® or nab-paclitaxel is the first clinically approved nanostructured polymer–drug conjugate (36). Compared to conventional paclitaxel administration, albumin-based nanoparticles demonstrated enhanced biocompatibility and tumor accumulation of paclitaxel (38). Another key advantage of this natural polymer was that it reduced the toxicity of paclitaxel to healthy tissues by altering the pharmacokinetics ultimately enhancing the anti-cancer therapeutic efficiency (38,39). At present, numerous clinical trials are underway for albumin-paclitaxel nanoparticles for the treatment of multiple cancer types (Table II).

Table II. Current clinical trials for albumin-paclitaxel nanoparticle conjugates

Company/sponsor	Drug/formulation	Conditions	Phase
City of Hope Medical Center	Paclitaxel-albumin	Male breast cancer, Recurrent breast cancer, and Stage IV breast cancer	II
Chinese Academy of Medical Sciences	Paclitaxel-albumin with nedaplatin	Uterine cervical cancer	II
The First Affiliated Hospital of Guangzhou Medical University	Paclitaxel-albumin with cisplatin	Non-small cell lung cancer	II
City of Hope Medical Center	Paclitaxel-albumin	Ovarian cancer, Peritoneal cavity cancer, and Unspecified adult solid tumor	I
Columbia University/ Celgene Corporation	Abraxane®	Bladder cancer	I and II
Univ. of Alabama/ Susan G. Komen Breast Cancer Foundation/ Daiichi-Sankyo Pharma Development/ Triple Negative Breast Cancer Foundation	Abraxane®/ Tigatuzumab	Breast cancer, Triple negative breast cancer, Stage IV breast cancer, and Metastatic breast cancer	II
Celgene Corporation	Albumin-bound paclitaxel (ABI-007)/gemcitabine	Metastatic pancreatic cancer	III

Data retrieved from National Institutes of Health (NIH) Web site (www.clinicaltrials.gov) on 20 October 2013

Recently, cyclodextrin-drug conjugates have been added to the translational nanodrug delivery system arsenal. Cyclodextrins are polymeric amphiphiles where hydrophilic and hydrophobic components assist in micelle formation serving to compartmentalize various drugs. Significantly, Schleup *et al.* (40) demonstrated the efficacy of IT-101, a cyclodextrin–camptothecin conjugate, in six preclinical tumor models. This polymer nanoparticle allowed extended release of the anti-cancer agent resulting in a significant enhancement of anti-tumor activity against LS174T and HT29 (colorectal cancer), H1299 (non-small cell lung cancer), H69 (small cell lung cancer), Panc-1 (pancreatic cancer), MDA-MB-231 (breast cancer), and TC71-luc (Ewing's sarcoma) cell lines. The study also demonstrated that IT-101 was able to overcome resistance to irinotecan, an anti-cancer drug, in MDA-MB-231, Panc-1, and HT29 tumors (40). Another preclinical study demonstrated significant anti-tumor activity of cyclodextrin-based polymer camptothecin (CRLX101) towards gastric adenocarcinoma (41). The anti-tumorigenic activity of CRLX101 was mediated by a decrease in expression of tumor biomarkers associated with hypoxia and angiogenesis, including vascular endothelial growth factor and CD31(41). Such biological information has been vital for the improved success rate of this polymer–drug conjugate nanoparticle in preclinical and clinical trials. CRLX101 is currently under investigation in multiple clinical trials for a myriad of cancers (Table III).

In recent years, researchers have gained an interest in developing novel polymer nanoparticle formulations with advanced properties such as stimulus-responsive drug delivery systems. Notably, some of these nanoparticle systems have already entered clinical trials (33). For example, magnetic nanoparticles show great promise as stimuli-responsive systems in cancer therapy. Nanotherm® therapy (MagForce Nanotechnologies) involves the direct administration of magnetic nanoparticles into the tumor followed by application of alternating currents (33). The nanoparticles have iron oxide cores coated with aminosilane and are responsive to changes in the magnetic field, which increases their polarity leading to the generation of heat at the tumor site. Thus, intratumoral heat destroys the tumors. Significantly, Nanotherm® therapy has received european regulatory approval for the use in glioblastoma patients.

Efficient translocation of drug molecules toward cancer tissue without affecting healthy tissue is a great challenge in cancer drug delivery. To address this issue, a pH-responsive nanoparticle system was developed by incorporating pH-

Table III. Active clinical trials of CRLX101

Conditions	Interventions	Phase
Advanced or metastatic stomach and Non-resectable gastro-esophageal cancer	CRLX101	I (pilot trial)
Advanced non-small cell lung cancer	Supportive care and CRLX101	II
Recurrent small cell lung cancer	Topotecan with CRLX101	II
Advanced solid tumors	Camptothecin conjugated to a linear, cyclodextrin-based polymer	I and II
Ovarian cancer, Fallopian tube cancer, and Primary peritoneal cancer	CRLX101	II
Advanced renal cell carcinoma	CRLX101 (Cerulean) with bevacizumab	I

Data retrieved from National Institutes of Health web site (http://clinicaltrials.gov) on 21 October 2013

responsive polymers in nanoparticles (42). The cellular uptake of these pH-responsive nanoparticles was remarkably efficient at both lower and higher pH ranges providing new insights into how stimulus responsive nanoparticle systems control the cellular uptake of nanoparticles. In yet another study, chitosan nanoparticles were used for pH-responsive drug delivery of tamoxifen in breast cancer cell lines (43). This study reported the ability of controlled drug delivery at a low (4.0 to 5.0) pH range, which is congruent with the targeted delivery of tamoxifen to a tumor microenvironment.

The combination of nanoparticles and biologically active components is of intense interest because of the synergistic properties being exploited by such novel technology. Specifically, non-anticoagulant heparin is currently under investigation as a drug delivery system for the treatment of cancer because of its known ability to inhibit angiogenesis and metastasis (44). For example, Wang *et al.* (45) recently demonstrated the potent anti-cancer activity of a non-anticoagulant heparin-conjugated nanosystem in an ovarian mouse model. In this system, succinylated heparin with a single amino acid spacer (leucine) was conjugated to paclitaxel, which formed the inner core of the nanoparticle. Subsequently, researchers were able to demonstrate tumor targeting using succinylated heparin with PEG 1000/3000 spacers conjugated to a taxol core and surface decorated with the targeting ligand folate (46). The folate-conjugated heparin-taxol nanoparticles were more efficiently taken up by folate receptor overexpressing cancer cells compared with folate receptor-deficient cell lines. Heparin has also been conjugated with chitosan (47), gold (48), and iron oxide nanoparticles (49). Moreover, heparin-based co-polymers have been used to develop multifunctional nanoparticle systems for anti-cancer drug delivery. Li *et al.* (50,51) explored the anti-tumorigenic activity of folate-conjugated heparin with poly(β-benzyl-aspartate). The co-polymeric nanoparticle system had amphiphilic character and was used for targeted delivery of doxorubicin. This delivery system demonstrated pH-dependent release of its cargo and showed potent cytotoxic effects against folate receptor-positive cancer cells. In other studies, dendronized heparin drug conjugates have been used for cancer therapy (52). Dendrimers are branched polymers that form tree-like nanoscaled structures. When complexed with heparin, the nanoparticle displayed pH sensitivity imparting controlled delivery of doxorubicin at acidic pH ranges in a mouse breast tumor model. The dendronized heparin-conjugated nanoparticle displayed potent anti-angiogenic and strong anti-tumorigenic activity (52).

NANOPARTICLE MODIFICATION APPROACHES FOR SPEEDY TRANSLATION

Preclinical data is invaluable for the successful design of a nanodrug delivery system to achieve clinical applicability. Nanoparticles must display reasonable circulation half-life with reduced renal clearance rate, exhibit no or limited toxicity to normal tissues, or poorly immunogenic, and have tumor-specific properties. The following modifications to nanoparticle fabrication are considered important for the speedy translation of nanodrug delivery systems.

Polyethylene Glycol Modification

The physicochemical characteristics of nanoparticles are important determinants of the fate of nanodrug delivery systems *in vivo*. The innate immune system readily recognizes and destroys foreign material that enters the body's circulation. In the absence of camouflage, nanoparticles interact with blood proteins that consequently results in clearance by the mononuclear phagocytic system (MPS), which drastically reduces the half-life of the nanoparticle. Additionally, phagocytic clearance results in the accumulation of nanoparticles and its cargo therapeutic in MPS organs, such as the liver and spleen, likely inciting excessive normal tissue toxicity (53). PEG modification by covalent or noncovalent linkage to a nanoparticle, however, is an intermediary step towards translating bench nanoparticle technology into a patient applicable therapeutic (54,55). PEG, a hydrophilic polymer with a neutral charge, forms a dense layer over the nanoparticle surface imparting necessary steric hindrance to avoid blood proteins from binding to the nanoparticle surface. This effectively delays phagocytic clearance and confers prolonged circulation time for the nanoparticles (52). Importantly, however, PEG is nonbiodegradable with the molecular weight of the PEG chains influencing its renal clearance from the body. Numerous studies have shown that PEG-modified nanoparticles remain in circulation for extended periods of time and are passively targeted to tumor tissues (56,57). Passive targeting utilizes the characteristic enhanced permeation and retention (EPR) effect of solid tumors. The fate of PEG-modified (stealthed) or non-PEGylated (conventional) nanoparticles after intravenous administration are shown in Fig. 1. Opsonin proteins in the circulation recognize non-PEGylated nanoparticles targeting them for rapid clearance from the circulation. Most of these nanoparticles are carried to the mononuclear MPS organs, such as the liver and kidney resulting in poor accumulation of nanoparticles in the tumor. By contrast, stealthed nanoparticles evade opsonin protein recognition for an extended period of time resulting in enhanced tumor accumulation. In a recent study, Choi *et al.* (58) investigated the tumor-targeting ability of PEGylated hyaluronic acid nanoparticles. The nanoparticles were fabricated through self-assembly of PEG and hyaluronic acid. These nanoparticles exhibited a negative charge in the physiological environment and displayed enhanced tumor uptake with a concomitant reduction in accumulation in the liver. Interestingly, the tumor uptake of PEGylated nanoparticles was approximately 1.6-fold higher than non-pegylated nanoparticles indicating affective extravasation of PEGylated nanoparticles into tumor tissues. Other studies have explored PEGylated nanoparticles for passive targeting of neuroendocrine tumors in a mouse model (59). Polycaprolactone/PEG (PCL/PEG) nanoparticles were loaded with octreotide, a somatostatin analog, at a high loading efficiency (66–84%) and administered to tumor-bearing mice. Enhanced tumor accumulation of PCL/PEG nanoparticles was observed compared with free octreotide suggesting that the accumulation of nanoparticles was most likely mediated through the EPR effect (56).

In addition to simple PEGylation, a wide variety of tumor-targeting ligands have been covalently coupled to the surface of nanoparticles via PEG chains, which act as spacers between targeting moieties and the nanoparticle surface. The

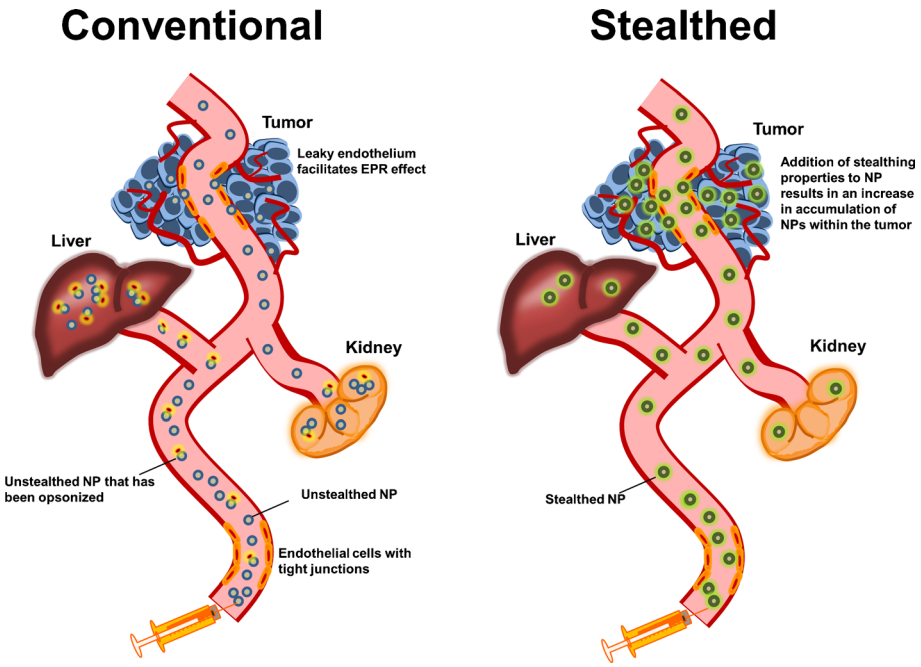

Fig. 1. Schematic showing stealthed nanoparticles are poorly recognized by opsonin proteins in circulation allowing nanoparticles to evade rapid phagocytic clearance resulting in enhanced tumor accumulation. By contrast, conventional nanoparticles that are not stealthed are rapidly opsonized and cleared from the circulation resulting in poor tumor accumulation

chain length plays an important role in determining the efficiency of ligand–receptor interactions. Recently, Kawano and Maitani (60) examined the effect of PEG spacer length and folate ligand density on tumor-targeting efficiency of folate-modified liposomes. Using PEG-DSPE-linked spacers of varying PEG molecular weights coupled with folate ligands, studies demonstrated that sufficiently longer PEG chains combined with low folate modification ratios resulted in enhanced folate–receptor binding in folate–receptor overexpressing human KB cells, a subline of the human cervical HeLa tumor cell line. Similarly, in another study a PEG spacer was used for folate conjugation onto PLGA nanoparticles (61). PEG chains coupled to thioctic acid and folate on opposite ends were conjugated to gold nanoparticles selectively targeting folate receptor positive cells (62). As a novel anti-tumor strategy, Hong *et al.* (63) proposed a transferrin-conjugated nanoparticle system for the delivery of hydroxyl camptothecin to *in situ* tumors. Using a sarcoma tumor (S180) model, the authors demonstrated that the blood retention time and targeting efficiency were increased by the PEG chain-conjugated transferrin-targeted nanoparticle loaded with hydroxycamptothecin (60). Taken together, these studies demonstrate that PEG chains can act as cross linkers to facilitate active targeting as well as increase the translational potential of nanoparticle drug delivery systems.

Tumor-Targeted Nanoparticles

Nanoparticles loaded with anti-cancer drugs or therapeutic genes can be targeted to tumor sites using targeting moieties. These ligands should be specific toward receptors overexpressed by cancer cells. Targeted drug delivery systems are known to enhance the therapeutic efficacy of their payload drug because of site-specific delivery and subsequent increase in tumor uptake of the therapeutic compared with nontargeted conventional drug delivery systems (64). At present, an extensive collection of targeting moieties are available including antibodies, peptides, oligonucleotides (aptamers), and small molecules such as folic acid, transferrin, and integrins (65).

Antibody-conjugated liposomes for tumor targeting have been used for over two decades. Hughes *et al.* (66) demonstrated the use of the first monoclonal antibody targeted liposomes for organ specific delivery in lung tumor-bearing mouse models. Since then several studies have used antibody-based targeting strategies for selectively targeting tumor-specific receptors and release of intracellular therapeutic payloads (67–69). Immunoliposomes are antibody-conjugated liposomes used for tumor-targeted drug delivery (70). Once localized via the EPR effect to the tumor site, the antibodies bind specific over-expressed cell surface receptors on the tumor cells promoting immunoliposome–tumor cell interactions resulting in enhanced intracellular delivery of the nanoparticle payload. Examples of targeting antibodies for tumor-specific antigens include anti-human epidermal growth factor receptor2 (HER2) (71), anti-EGFR (72), anti-CD19 (73), and GAH ($F(ab)_2$ goat anti-human monoclonal antibody), which recognizes a tumor specific epitope that is not yet well characterized (74). However, it has been reported that blood clearance of whole antibody-liposome conjugates occurs more readily than liposomes conjugated with Fab′ antibody fragments, which lack the Fc region of an antibody (75). Membrane type-1 matrix metalloproteinase (MT1-MMP), which is expressed in tumor endothelial cells, plays an important role in angiogenesis. Hatakeyama *et al.* (76) used MT1-MMP targeting Fab′ conjugated immunoliposomes for targeted delivery of doxorubicin to an orthotopic fibrosarcoma mouse model. Cellular

uptake was accelerated for anti-MT1-MMP (Fab′)-liposomes compared with nontargeted liposomes. However, overall tumor accumulation of both the liposomes was comparable and favored by the EPR effect (76). Researchers have also constructed anti-EGFR-conjugated liposomes for co-delivery of adriamycin and radionucleotide reductase M2 siRNA to achieve combined therapeutic effect toward orthotopic hepatocellular carcinoma-bearing mouse models (77). Efficient EGFR targeting resulted in enhanced tumor growth inhibition. Recently, a phase I clinical trial utilizing an anti-EGFR immunoliposome targeted to solid tumors was successfully completed (72). This immunoliposomal doxorubicin was found to be well tolerated up to a dose of 50 mg/m^2. Phase II clinical studies are anticipated for this formulation.

Nanobodies® (Ablynx) are synthetic proteins that are derived from and possess functional properties of an antibody. Structurally, nanobodies lack the light chains of an antibody but have a heavy-chain domain with a single variable domain and two constant domains (CH2 and CH3). This configuration facilitates the retention of the full antigen-binding capacity similarly to the parental antibody (78). Nanobodies are very stable and combine the advantages of an antibody and a small molecule drug and exhibit high target specificity. Additionally, they have also been recognized as an important tool for *in vivo* imaging (79). Nanobodies have been used in the novel development of a noninvasive imaging technique to accurately identify overexpressed surface receptors on tumor cells. Recently, nanobodies were used to screen for HER2 in a breast cancer model (80). The nanobodies bound specifically to HER2-expressing cells and mouse xenograft tumor models and showed low accumulation in nontarget organs as revealed by single-photon emission computed tomography. Preliminary studies showed therapeutic benefits of nanobodies, including efficient blocking of EGF-mediated signaling and inhibition of EGF-induced cell proliferation (81). The advent of nanobodies thus permits rapid development of anti-cancer nanobody-based therapeutics.

Aptamers are short oligonucleotide sequences that bind to a target with high specificity and possess advantages, such as small size (~15 kDa) and poor immunogenicity. Nanoparticles decorated with aptamers hold great promise in targeted drug delivery and imaging. AS1411, a nucleolin-targeted DNA oligonucleotide, is the first aptamer to be investigated in a phase II clinical trial for its tumor-targeting property (82). In a recent study, AS1411-conjugated PLGA-lecithin-PEG nanoparticles were used for site specific targeted delivery of an anti-cancer drug (83). The nanoparticles showed enhanced cellular uptake and cell killing efficiencies compared with nontargeted nanoparticles demonstrating the efficiency of AS411 aptamers in targeted therapy. In other studies, Mann *et al.* (84) demonstrated the affinity of thiolated oligonucleotide aptamer (thioaptamer) towards E-selectin overexpressing tumor cells. These thioaptamer decorated liposomes were injected into a mouse tumor model to study their accumulation in the tumor vasculature. Importantly, conjugation of the thioaptamer resulted in selective targeting and binding of the stealth liposome towards E-selectin overexpressing tumor vasculature. Thus, aptamers have great potential in clinical translation of nanodrug delivery systems because of their ability for specific targeting and inhibition of protein function as a result of their protein-binding properties. Detailed information on aptamer-based therapeutic systems for various therapies including cancer has been recently reviewed elsewhere (85).

Tumor-targeted liposomal carriers can also be used to improve the delivery of gene therapeutics. Camp *et al.* (86) reported that anti-transferrin receptor (TfRscFv) targeted liposomes were able to specifically deliver wild-type human *p53* tumor suppressor genes (SGT-53) in a metastatic pancreatic mouse model. In combination with gemcitabine, survival rates of mice with metastatic tumors were significantly improved. Importantly, such preclinical studies have propelled the induction of multiple phases I and II clinical trials investigating targeted-liposome-*p53* complexes (86,87). In one such trial, the SGT-53 was targeted using the anti-transferrin receptor to deliver *p53* and restore tumor suppressor function in patients with advanced solid tumors. SGT-53 was well tolerated and resulted in transgene accumulation within metastatic tumors as well as demonstrated anti-tumorigenic activity (76).

Affibodies are small alpha-helical polypeptide ligands that function as inert antibody mimetics that have gained attention for their use in targeted delivery of nanoparticles. A recent study reported that polymeric nanoparticles conjugated with anti-HER2-targeted affibodies loaded with paclitaxel had enhanced *in vitro* cellular uptake resulting in a significant improvement of paclitaxel-induced cytotoxicity in ovarian, breast, and pancreatic cells (88). Arg-Gly-Asp (RGD) peptide, which selectively targets alphaVbeta3 ($\alpha_v\beta_3$) integrins on tumor endothelial cells, have increasingly been used for targeted anti-cancer nanodrug delivery (89). Targeting tumor vascular endothelial cells in mice bearing ovarian tumors using RGD peptide-conjugated chitosan nanoparticles resulted in enhanced delivery of therapeutic siRNA and significant tumor growth suppression (89). Conjugation of RGD peptides to nanoparticle surfaces increased the selective intratumoral delivery of the nanoparticle payload (90). In another example of peptide targeting, Cys-Arg-Gly-Asp-Lys (CRGDK) was designed for targeting Neuropilin-1 (Nrp-1) receptors, which are overexpressed in some cancer cells (91). Gold nanoparticles conjugated with CRGDK peptides were targeted for Nrp-1 receptor expressing MDA-MB-231 breast cancer cells. It was observed that administration of these gold nanoparticles resulted in enhanced cellular uptake of the nanoparticles and improved the delivery of a therapeutic peptide (91). Agemy *et al.* (92) used a targeted anti-angiogenic tumor-homing peptide CGKRK (Cys-Gly-Lys-Arg-Lys) nanoparticle system for selective delivery of pro-apoptotic peptides to glioblastoma multiforme (GBM)-bearing mouse models. Systemic delivery of the nanoparticle system resulted in eradication of most tumors in one mouse model and significant delay of tumor development in another GBM mouse model. Subsequently, they constructed a CGKRK pentapeptide-conjugated nanoparticle to target p32, which is expressed on both tumor cell surface and mitochondrial membranes, in an orthotopic breast cancer-bearing mouse model (93). This strategy selectively delivered the nanoparticle system to tumor associated cells and its payload to the mitochondria. Collectively, these studies strongly suggest the potential use of tumor-homing peptide targeted nanoparticles for translational clinical investigation.

TRANSLATIONAL APPROACHES IN NANOPARTICLE-BASED CANCER IMAGING

In vivo preclinical imaging is an essential tool for translational studies. Recently, there has been an emerging interest among cancer researchers towards the utility of antibody-conjugated nanoparticles in cancer imaging. For example, Kelley *et al.* (94) developed a tumor-targeted imaging agent for the detection of pancreatic ductal adenocarcinoma (PDAC), a malignancy with poor prognosis. Notably, magnetic resonance imaging (MRI) of small PDAC and precursor lesions in mouse tumor models was possible using Plectin-1-targeted peptide-conjugated magneto-fluorescent nanoparticles (94). In a different study, HER2 antibody-conjugated polymer-coated superparamagnetic iron oxide nanoparticles were used to effectively detect HER2-positive breast cancer cells (95). Additionally, Oghabian *et al.* (96) developed a MRI protocol aimed at detecting small populations of tumor cells using HER2-conjugated iron oxide nanoparticles. Other imaging modalities such as affibody-conjugated nanoparticles have also been explored in orthotopic mouse models. For example, a near-infrared magnetic iron oxide nanoparticle labeled with HER2-affibodies has been used for 2D optical imaging, 3D fluorescence tomography, MRI, spectroscopic imaging, and photoacoustic imaging of an ovarian tumor-burdened mouse model (97). Histological examination of nanoparticle-treated tumor samples demonstrated specificity of the anti-HER2 affibody-conjugated nanoparticle. Importantly, researchers were able to achieve multi-modality tumor imaging with systemic delivery of a single targeted nanoparticle system. Advancements in translational approaches such as improved sensitivity and selectivity of tumor imaging may enhance the clinical acceptability of affibody-targeted nanoparticles.

Qdots are semiconductor nanoparticles used for fluorescent probing of cancer cells. Targeting Qdots towards cancer cells has the potential to significantly increase the specificity of tumor imaging. Zhang *et al.* (98) reported the use of GS24 aptamer and T7-targeted peptide conjugated to CDSe/ZnS Qdots for fluorescent probing of cancer cells. The GS24 aptamer and T7 peptide targeted the mouse and human transferrin receptors, respectively, and greatly enhanced the image specificity. In other studies, researchers have developed a protocol for the use of RGD peptide-conjugated PEG-coated Qdots for tumor vasculature targeting (99). To date, however, no Qdot products have entered the clinic due to their inherent toxicity. Biofunctionalization of Qdots is an active area of research; however, it is beyond the scope of this review. Future directions should aim to develop reliable and specific delivery strategies of Qdots to exploit their full imaging capabilities and expedite the translational process.

Multifunctional Nanoparticles as Translational Tools

Multifunctional nanoparticles are capable of simultaneously targeting, imaging, and delivering drug payload. Numerous examples are currently available in the literature pertaining to the use of multifunctional nanoparticle systems in cancer drug delivery and imaging, such as liposomes, solid lipid nanoparticles, polymeric nanoparticles, Qdots, iron oxide nanoparticles, gold nanoparticles, dendrimers, micelles, and carbon nanotubes (100). Other than the encapsulated chemodrugs or therapeutic genes, a wide range of molecules, such as targeting ligands, stealth providing molecules, and image contrast agents, are linked to the nanoparticle to make it multifunctional (Fig. 2). For example, Aurimune® (CYT-6091), the first multifunctional nanoparticle system to enter the clinic, has both imaging and therapeutic functionalities (101). Aurimune® is composed of a colloidal-gold nanoparticle conjugated to the tumor growth inhibitor tumor necrosis factor alpha to achieve theranostic properties.

Recently, Eliasof *et al.* (102) constructed a nanoparticle system composed of a cyclodextrin backbone with covalently linked PEG polymer and camptothecin linked via a spacer molecule (CRLX101). CRLX101 targeted to solid tumors resulted in enhanced tumor uptake and controlled release of camptothecin over several days to inhibit tumor growth. Correlating preclinical and human clinical trial data demonstrated similar mechanisms of action in both suggesting that the behavior of CRLX101 in preclinical models is translatable to humans. Sahoo *et al.* (103) developed a dual thermo- and pH-responsive polymeric magnetic nanoparticle system modified with tumor-targeting ligands. The doxorubicin-encapsulated nanoparticle system was fabricated using dual-responsive poly(*N*-isopropylacrylamide)-block-poly(acrylacid) co-polymer coated over magnetite core. The nanoparticle was surface decorated with the tumor targeting ligand folate as well as conjugated for flourescent labeling with Rhodamine B isothiocynate. Intracellular uptake of this nanoparticle was monitored using fluorescence microscopy. Furthermore, this smart nanodrug delivery system was capable of targeted stimulus-responsive release of doxorubicin inciting significant cytotoxicity toward the human cervical cancer cell line HeLa (103). Multifunctional nanoparticles are thus expected to overcome some of the conventional obstacles in cancer drug delivery and imaging with an ultimate aim to improve patient care. Multifunctional nanodrug delivery systems offer numerous advantages. However, the increased complexity rendered by addition of several functional modalities may result in issues related to toxicity, manufacturing, and scalability of the final product. Multifunctionality may also provide the nanoparticles with unpredictable behavior *in vivo* and ultimately more regulatory barriers for their clinical translation. The challenges for multifunctional nanoparticles for effective translation include the reproducibility in the synthesis and purification procedures, identification of appropriate ligands for targeting, and determination of the optimal ligand density on the particle surface (104). Another challenge posed is the charge- related toxicity contributed by one or more components used in the fabrication of multifunctional nanoparticle. For example, cationic lipids and polymers are known for their toxicities when interacting with biological components (105,106). By contrast, biologically inert materials while enhancing safety are often less bioactive (107). Purification and sterilization of nanoparticles with multiple chemical entities poses another important challenge. In general, particles are purified by repeated centrifugation, centrifugal filtration, or syringe filtration. Centrifugation is a simple and effective method of purifying the particles; however, if the particles are not ligand-stabilized, chances of aggregation are much higher. Filtration often results in loss of the nanoparticles by sticking to the membranes,

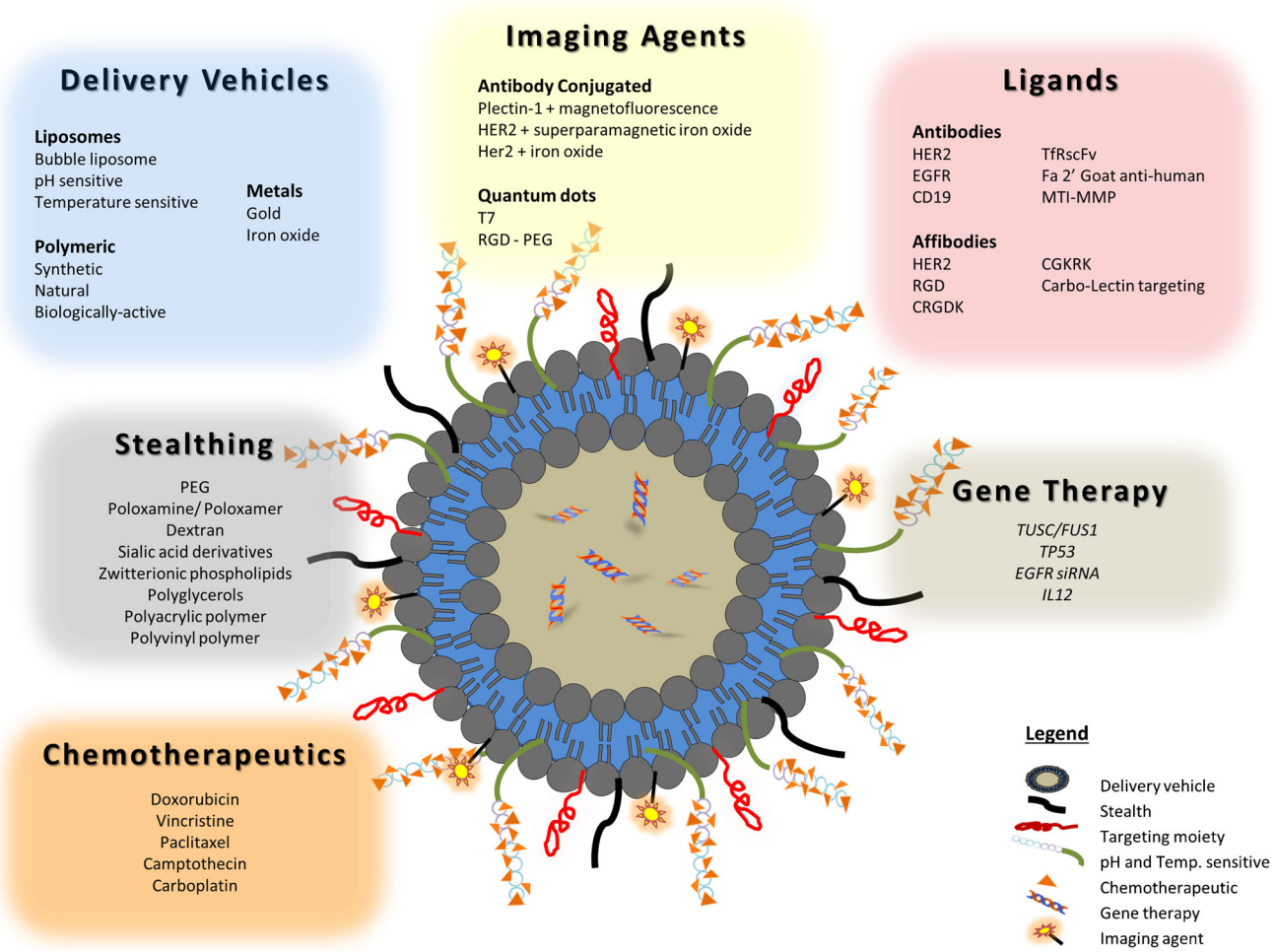

Fig. 2. Multifunctional nanoparticle systems can be designed with a choice of drug delivery vehicle, tumor-targeting ligands, molecular imaging agents, and biological and synthetic therapeutics and cloaked with stealth properties. The schematic lists an abbreviated menu of currently available preclinical options to compose novel nanoparticle systems

whereas dialysis and chromatographic techniques are time consuming but known to preserve particle integrity during purification procedures. Additionally, ligand density on the nanoparticle surface is an important factor that determines the efficiency of specific targeting (105). An improper balance between ligand density and stealth property may lead to rapid clearance of the nanoparticles from the circulation. Weak ligand–receptor interactions may also occur given the dynamic nature of tumor receptor expressions. Therefore, adding multiple-targeting ligands to the multifunctional nanoparticle is an important strategy to overcome weak affinity of some ligand–receptor interactions. Ultimately, however, it is imperative to employ a cautious approach in the development of multifunctional nanoparticles for translational studies.

EMERGING TECHNOLOGIES MAY ACCELERATE TRANSLATION OF NANOPARTICLES

The ability of researchers to manipulate small particles on a fine scale has remained a challenge in the nanotechnology field. Recently, however, application of novel technologies such as microfluidics or nanofluidics and biomimetics has emerged actuating drug delivery strategies. Microfluidics involves the engineering of a system to manipulate with high precision ultra-small volumes, such as nanoliters of liquids (108). Microfluidic technology will facilitate screening of nanodrug delivery systems in a high throughput fashion for well-controlled, batch-to-batch reproducible fabrication of nanoparticles and assess their biological behavior for biomedical applications (109). Using microfluidic technology, Majedi *et al.* (110) synthesized monodispersed-chitosan nanoparticles via self-assembly. The nanoparticle system demonstrated high tunability of its drug release profile supporting the feasibility of microfluidic technology in the large scale fabrication of nanodrug delivery systems.

Biomimetics deals with the design and implementation of nanodevices that replicates a biological system or entity. For this reason, biomimetic polymer drug delivery systems with amphiphilic properties have attracted immense interest in anti-cancer drug delivery. Interestingly, in a recent report, a polymer nanoparticle was disguised with red blood cell (RBC) cellular membranes in order to evade immune-mediated clearance and effectively deliver it's anti-cancer therapeutic (111).

Cloaking the nanoparticle with RBC cellular membranes substantially increased the circulation time of the nanoparticle system. Another study recently reported the use of outer cellular membrane mimetic structures for fabrication of co-polymer micelles with amphiphilic character (112). The phospholipid micellar drug delivery vehicle consisted of the anti-cancer drug adriamycin incorporated into crosslinkable amphiphilic co-polymers such as 2-(methacryloxyethyl) phosphorylcholine, stearyl methacrylate, and trimethoxysilylpropyl methacrylate. This biomimetic micelle showed remarkable sustained drug release behavior with no burst effect (112). Clearly, microfluidics and biomimetics offer novel strategies to supersede the historical challenges faced by nano-scientists to rapidly excel the translation of nanodrug delivery systems into the clinical setting.

MITIGATING TRANSLATIONAL ISSUES IN NANODRUG DELIVERY SYSTEMS

Clinical translation of advanced drug delivery and imaging systems present challenges despite the enormous potential they display in preclinical studies. Targeted nanodrug delivery systems are more complex than simple nanoparticle systems. This has raised concerns associated with reproducibility and long-term stability of such nanoparticle systems. Expediting clinical trials with such issues further complicates the validation process and is an ethical and regulatory related concerns. Additionally, nanoparticle handling and pre-analytical variability pose serious challenges with the reliability and reproducibility of preclinical data to translate into human clinical trials. Furthermore, a better understanding of the interaction between the nanoparticle and tumor microenvironment, especially with tumor cells, is necessary. Elucidation of the variation in cellular trafficking observed between different nanoparticles and cell types will provide a deeper understanding of nanoparticle internalization and physiologically relevant nanoparticle behavior.

Safety and quality of nanoparticle systems are important concerns for health care applications. Toxicity issues may arise from a poor understanding of nanoparticle physicochemical properties, their biological behavior, and wrong prediction of dosage regimens. Lack of specific standard testing and characterization protocols are also important concerns for a plethora of nanoparticle formulations with dynamic properties (4). Given the challenges encountered, development of uniform regulatory guidelines would significantly aid in the improvement of preclinical safety evaluation and potentially expedite the delivery of clinically relevant nanoparticle systems to the clinic setting. Advancements in technologies such as microfluidics will likely aid researchers in the development of much needed reproducible, large scale screening procedures for nanoparticles in biologically relevant systems.

CONCLUSIONS

The field of nanomedicine has already made significant advancements, including the clinical approval of a number of nanodrug and imaging delivery systems in cancer therapy. However, challenges of complexity and toxicity bottleneck the translation of a large group of novel nanodrug delivery systems. It is important to note that multiple barriers exist before a nanodrug delivery system can enter into the clinical setting. The nanoparticle carriers should qualify all the routine standards for a pharmaceutical system such as safety, quality, stability, and bioavailability. In addition proper knowledge of nanoparticle structural components, preparation and sterilization methods, reproducibility, and biological interactions are necessary to ensure a successful preclinical evaluation. Currently, the key step needed to bridge laboratory nanoparticles to therapeutic application is setting proper regulatory standards for routine monitoring and evaluation of nanoparticles. Harmonized efforts between researchers, clinicians, pharmaceutical companies, and regulatory authorities are required to achieve the goal of speedy translation of nanodrug delivery systems. It is anticipated that advancements in novel technologies will aid in overcoming these challenges to enhance the clinical applicability of nanodrug delivery systems for use in cancer therapy.

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