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Image-Guided Nanosystems for Targeted Delivery in Cancer Therapy

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Abstract

Current challenges in early detection, limitations of conventional treatment options, and the constant evolution of cancer cells with metastatic and multi-drug resistant phenotypes require novel strategies to effectively combat this deadly disease. Nanomedical technologies are evolving at a rapid pace and are poised to play a vital role in diagnostic and therapeutic interventions - the so-called “*theranostics*” – with potential to advance personalized medicine. In this regard, nanoparticulate delivery systems can be designed with tumor seeking characteristics by utilizing the inherent abnormalities and leaky vasculature of solid tumors or custom engineered with targeting ligands for more specific tumor drug targeting. In this review we discuss some of the recent advances made in the development of multifunctional polymeric nanosystems with an emphasis on image-guided drug and gene delivery. Multifunctional nanosystems incorporate variety of payloads (anticancer drugs and genes), imaging agents (optical probes, radio-ligands, and contrast agents), and targeting ligands (antibodies and peptides) for multi-pronged cancer intervention with potential to report therapeutic outcomes. Through advances in combinatorial polymer synthesis and high-throughput testing methods, rapid progress in novel optical/radiolabeling strategies, and the technological breakthroughs in instrumentation, such as hybrid molecular and functional imaging systems, there is tremendous future potential in clinical utility of theranostic nanosystems.

Keywords

Drug delivery; image-guided delivery; liposomes; multifunctional nanosystems; personalized medicine; polymeric nanoparticles; theranostics; tumor targeting

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CONFLICT OF INTEREST

None declared.

1. INTRODUCTION

1.1. Cancer Challenges

The diagnosis and treatment of many cancers stills remains elusive and a major barrier to effective clinical outcomes. A recent review by Hanahan and Weinberg [1] elucidates the “hallmark” characteristics of cancers and the deregulation and signaling pathways that are involved in the development and progression of tumors. Cancer cells are known to possess unique characteristics of dividing and proliferating rapidly, resisting cell death or apoptosis, and having devastating invasive potentials. Also, tumor cells have the ability to infiltrate surrounding normal tissues or penetrate into lymphatics and/or blood vessels enabling them to circulate (called circulating tumor cells) and migrate to multiple distant sites or organs, forming secondary tumors [1, 2]. This process called “tumor metastasis” often occurs at an advanced stage of the disease and is generally beyond the scope of surgical resection or other forms of treatment, largely responsible due to the failure in early detection and management of the disease. Even in the case where surgical resection is a viable option, there is always a high risk of relapse of the disease due to existence of microscopic subpopulations of recalcitrant tumor cells that are speculated to possess “stem cell like” characteristics [3], that play a key role in self-renewal and formation of new types of tumors [4]. Apart from these inherent destructive qualities, tumor cells have the capability to reprogram themselves to suite various demanding and testing conditions of the tumor microenvironment, such as the ability to survive (and often thrive) in highly acidic pH [5, 6] and very low oxygen or hypoxic conditions [7–10]. Moreover, most tumors cells acquire resistance or become insensitive to a broad spectrum of structurally and functionally different (and otherwise effective) anticancer agents on prolonged exposure; a process known as acquired tumor multi-drug resistance (MDR) [11–13]. The micro-environmental selection pressures that leads to the acquired MDR phenotype is well known to be a major reason for drug treatment failure in cancer patients [14]. Also, the genomic instability in cancers gives rise to accumulation of mutations and this instability leads to intratumoral (i.t.) heterogeneity in successive generations of tumors [15]. These ever-changing and unpredictable genetic mutations in cancers [16] make them a tricky target to pursue, and a major hurdle in the successful management of this malignant disease.

1.2. Systemic Delivery Challenges

Most of the conventional treatments for the management of cancers involve chemotherapy using low molecular weight anticancer agents that are systemically administered by the intravenous (i.v.) route. This intervention inherently lacks the ability to target tumors selectively. The drug indiscriminately diffuses across blood vessels into normal tissues and tumors alike, not only causing acute and often dose limiting side effects to cancer patients [17, 18] but also effecting in dilution of the drug, wherein the percent-injected dose that usually reaches the tumor is often very low, leading to suboptimal drug utilization and efficacy [19]. Low molecular weight anticancer compounds also suffer from short plasma circulation half-lives due to their rapid clearance through renal excretion. Also naked proteins, peptides, DNA, small interfering RNA (*siRNA*), and therapeutic molecules that are administered *via* the blood stream are generally labile and prone to degradation in the

presence of serum or plasma components [20, 21]. These shortfalls make systemic delivery of drugs and genes a great challenge.

1.3. Role of Molecular Imaging in Cancer

Many cancers can be prevented, better managed, or even cured if the pathological tissue is detected early and the progression of the disease can be effectively monitored through physiological rather than anatomical changes [22, 23]. In this regard, molecular imaging using targeted contrast agents, such as nuclear and optical probes, can play a leading role in screening, detection, and management of cancers [24, 25]. Imaging the molecular features or signatures of cancer cells/tissues can also serve an important clinical function [26–28]. For instance, molecular imaging using radiolabeled ligands, antibodies, or peptides that can target/bind to molecular markers, receptors or over-expressed targets on specific tumor cell surfaces can not only help locate such tumors (Fig. 1) [29], but also be used to study the biological effects of such molecules in the progression of the disease [30]. Also, imaging the downstream markers or effector molecules that signal cell death or apoptosis can provide indicators of treatment response much earlier than physical changes start to appear, such as tumor shrinkage [31]. Thus, molecular imaging of cancers can afford a gamut of meaningful information that can be applied for mechanistic understanding of the disease [32] as well as early detection [33], and for studying multiple events, such as non-invasive visualization of accumulation/localization of the nanoparticles *in vivo*, its biological fate, visualizing the progression/regression of the disease [25], and quantifying the effect of drug treatment in individual patients that usually vary on a case-by-case basis. Molecular imaging technology as applied to a cancer therapy is thus heralded to play a major role in the era of personalized medicine [34].

2. MOLECULAR IMAGING SYSTEMS IN CANCER

As discussed in the above section, the role of imaging in characterization of cancers at the molecular level would have major clinical implications, especially, for early detection of the disease, predicting the risk of tumor formation from precancerous lesions and ways to manage and treat the disease, including development of new molecular target based therapeutics [35]. In this regard, imaging systems have already been an integral part in screening, diagnosis, and staging of many cancers [28]. Among them, magnetic resonance imaging (MRI) has been a popular tool in tumor detection because of its high depth penetration, spatial resolution and high soft tissue contrast [36]. More importantly, MRI-based imaging circumvents the use of harmful ionizing radiation [37]. A significant amount of research in the field of MRI has been focused on the development of contrast agents to improve image signal intensity, and among them Gd^{3+} -based MRI agents have been most successful [38, 39].

X-ray computed tomography (CT) has also been used extensively for screening, detection, and measuring the progression of cancer in clinics [40]. In the conventional setting iodinated contrast agents are generally used to enhance the contrast for CT imaging. One such example is the intraarterial (i.a.) administration of the first commercially available polymer conjugated anticancer agent, SMANCS that is given to patients using iodinated lipid contrast

agent (Lipiodol®). SMANCS-Lipiodol® serves both as a diagnostic tool and a drug for therapeutic use; the contrast agent helps detect the accumulation of SMANCS in solid tumors and also facilitates imaging/monitoring the regression of tumors based on X-ray CT [41]. It has been reported that the radiation exposure to patients while undergoing CT scans in the past may itself serve as a source of cancers [42], however with the advancement in novel imaging technologies such as low-dose CT, the mortality from cancers could not only be reduced but the screening also resulted in detection of many tumors at early stages [43].

Among the nuclear modality imaging, positron emission tomography (PET) and single photon emission computed tomography (SPECT) having high detection sensitivity has been the most sought, for staging and follow up of solid tumors in patients in the clinics [44, 45]. Also, the vast choice of radionuclides and versatility of conjugation chemistries available for radiolabeling are yet another key advantage with nuclear modality imaging [46, 47]. Another prominent feature of nuclear imaging is its ability to image biological processes and metabolic activity in tissues and organs, e.g., PET using the analog of glucose 2-[¹⁸F]-fluoro-2-deoxy-D-glucose (¹⁸F-FDG) containing the ¹⁸F isotope with a moderate half-life $\approx$ 110 min and high-energy positron emission (0.6335 MeV) [48], is routinely used to detect tumors [49]. Solid tumors usually have higher uptake of ¹⁸F-FDG due to relative higher levels of glucose transporters and the action the enzyme hexokinase [50]. The average FDG PET sensitivity and specificity across all oncology applications is estimated at 84 % [51]. However, the production of positron emitting PET nuclides generally requires a dedicated and costly cyclotron in close proximity to the imaging facility due to the short half-lives of radionuclides. It is for this reason that radiolabeling drugs/molecular markers with PET-radiotracers are difficult to produce and image within the time frame of their decay [52]. ⁶⁸Ga is a valuable alternative to ¹⁸F for PET imaging because it does not need an on-site cyclotron and also because of its high positron emission of 1.899 MeV [52, 53].

Another alternative to PET, is the use of SPECT imaging. Although SPECT generally has lower radionuclide detection efficiency over PET, recent advancements in multi-aperture pinhole SPECT technology significantly reduces the difference in detection efficiencies between both modalities [54]. ^{99m}Tc is a versatile radioisotope for SPECT imaging that emits readily detectable 140 KeV γ -rays with a half-life $\approx$ 6 h [55]. The time frame of its decay is ideally suited for labeling for *in vivo* setting because it is long enough for scanning with SPECT instrument but at the same time keeps the radiation exposure low and helps reduce radiation burden to patients [55]. ^{99m}Tc is thus currently the most commonly used isotope for disease diagnosis, especially for cancers. Gamma emitting SPECT nuclides such as ¹¹¹In, having longer half-lives, could be advantageous in imaging pharmacokinetics and tissue biodistribution of radiolabeled drugs in small animal models, using micro-SPECT [56–58].

In the past few decades, along with the increased interest in novel nanoprobe development, there has been a burgeoning growth in the development of cross-functional imaging technologies that are revolutionizing the field of biomedical imaging and nanotechnology. For instance, CT or MRI that can provide structural information are being combined with radionuclide imaging techniques such as PET/SPECT or optical imaging that lacks these features [59]. Some such examples include the clinical hybrid/fused PET-CT and SPECT-CT

[60], and preclinical small animal micro-PET/SPECT-CT imaging systems, which has the ability to co-register both functional and anatomical features previously lacking in SPECT or PET alone [61]. In this regard, it is important to note that the breakthrough in preclinical small animal imaging systems have helped to carry forward the advances made in translational research into clinical practice [61, 62]. Other combinations of functional and structural imaging modalities such as PET/MRI and optical/MRI are also under development for both preclinical and clinical imaging [63].

Among the non-radioactive imaging tools, optical imaging using fluorescently labeled drugs/molecular markers is becoming very attractive and promising for *in vitro* screening as well for *in vivo* preclinical/clinical assessments [64–66]. Nevertheless, the application of fluorescence imaging for detecting cancers was demonstrated as early as 1948 by Moore *et al.* using sodium fluorescein as the fluorophore [67]. In recent times, the two most commonly utilized optical imaging techniques are based on 2D-fluorescence reflectance imaging (FRI) and 3D-fluorescence molecular tomography (FMT) imaging. The 3D tomographic imaging using FMT is in a way similar to CT scans but without the use of ionizing radiations [68].

In the current scenario of molecular marker-based cancer screening/identification and detection, fluorescence imaging is proving to be an indispensable tool for rapid assessment. Furthermore, the commercial availability of wide range of near-infrared (NIR) optical probes, the relative ease of photo-labeling and development of reliable chemical conjugation chemistries, coupled with low cost and relative easy access to optical imaging stations makes them a preferred choice for molecular imaging [65]. However, fluorescence imaging also have their own problems and pitfalls such as low sensitivity due to low penetration of fluorescent light in tissues, rapid bleaching or quenching of the fluorophore, and auto fluorescence (or false positive background signal) from soft tissues. Irrespective of these limitations, optical imaging based (nano)-probe development is moving in the right direction with many product pipelines in preclinical development [69].

3. MULTIFUNCTIONAL NANOPARTICLE DELIVERY SYSTEMS FOR CANCER

3.1. Polymeric and Liposomal Nanoparticulate Formulations

Drugs/genes encapsulated in nanoparticles offers several advantages over conventional treatment regimens in the management of cancers [70, 71]. As discussed earlier, chemotherapy involving systemic administration of a free drug (often a low molecular weight anticancer compound), suffers from many drawbacks, such as short plasma half-lives (necessitating high dose and frequent drug administration) and severe systemic toxicity due to distribution in non-target sites. Apart from these limitations, most of the anticancer drugs are hydrophobic in nature and, therefore, insoluble or poorly soluble in water, which makes it challenging to administer an adequate therapeutic dose. Encapsulation of such molecules in nanoparticulate formulations using biodegradable and biocompatible polymers or liposomal delivery systems can increase the drug payload, protect the encapsulated drugs from degradation in the bloodstream and also prolong their half-lives several folds [72–74]. Thus nanoparticulate drug formulations necessitate less frequent dose administration to cancer patients, thereby greatly improving the patient's quality of life.

Additionally, in the conventional therapeutic approach, the only way to achieve desired clinical outcome is by waiting for tumor regression after treatment. It is impossible to know if the drug has actually reached the tumor mass or remains available for sufficient duration to induce the tumoricidal effect. In this regard, image-guided therapeutic delivery using nanoparticles offers great potential in determination of drug availability at the target site as well as monitoring the therapeutic effect of the encapsulated payload in real-time with specific information on individual patients rather relying on the averages for clinical decision making [75].

3.2. Passive and Active Targeting to Cancers

It is well known that tumor cells possess devastating invasive potentials and divide and multiply at phenomenal rates. In order to cater to the ever-increasing oxygen and nutritional demands of the growing tumors, cancer cells start “neovascularization” or the formation of new blood vessels. This phenomenon of “angiogenesis” then starts to feed the rapidly growing tumor mass [76]. The tumor microenvironment and angiogenic characteristics of solid tumors are thus found to be very different from normal tissues. For instance, the tumor blood vessels are often aberrant or “leaky” [77]. The endothelial cells are disorganized or poorly aligned with large fenestrations and the perivascular cells and basement membrane are frequently abnormal or totally absent in the vascular wall. Furthermore, tumor tissues have a wide lumen and often lack the lymphatic clearance system [78–80]. It was found that the anatomical defectiveness and the functional abnormalities of the tumor blood vessels could be used to deliver macromolecular anticancer drugs selectively to solid tumor tissues - a phenomenon called the “enhanced permeability and retention” (EPR) effect of macromolecular drugs (Fig. 2), discovered by Matsumura and Maeda more than two and a half decades ago [80].

Most solid tumors have elevated levels of vascular permeability mediators such as vascular endothelial growth factor (VEGF), matrix metalloproteinases (MMPs), prostaglandins (PGs), bradykinin (BK), nitric oxide (NO), and peroxynitrite [81–84]. The overproduction of the vascular permeability mediators and the defective vascular architecture leads to extensive leakage of blood plasma components, lipid particles, and macromolecules into the tumors interstitium [85]. Furthermore, the slow venous return in the tumor tissues and the poor lymphatic drainage system helps retain the macromolecules for extended times periods while the extravasations into the tumor tissues continues [86]. It is thus possible to achieve very high local concentration of the macromolecular drugs in the tumor tissues. Drugs and genes encapsulated in macromolecular delivery systems such as liposomes, polymeric nanoparticles, and micelles are thus able to take advantage of this unique phenomenon to passively target tumor tissues [87, 88]. The EPR effect is now considered as a guiding principle for passive tumor targeting using polymer drug conjugates and nanoparticles [86, 89].

In general, macromolecules above the renal excretion threshold (typically above 40 kDa) are able to circulate for long periods of time in the blood and accumulate in solid tumors [87]. Also, liposomes, polymeric micelles and nanoparticles in the size range of 20–200 nm have been shown to extravasate and accumulate effectively in the tumor tissues [90]. This is

because the vascular pore size in the solid tumors can range from 200 to 600 nm [91]. The EPR effect will be optimal if the nanosystems can evade the reticuloendothelial system (RES) and show prolonged circulation half-life in the blood. In this regard, grafting a poly(ethylene glycol) (PEG) chain to the nanosystems can provide stealth characteristics and allow RES escape thereby rendering long plasma circulation half-life. For example, in the clinical setting doxorubicin loaded in PEG-grafted liposomes demonstrated enhanced tumor accumulation of doxorubicin and reduced toxicities compared with the free form of the drug [92].

Passive targeting can be a gateway to deliver drugs and imaging agents to many of the vascularized solid tumors, however, the delivery to avascular, hypovascler, or necrotic regions of tumors still remains a challenge [93]. In this regard, active targeting using antibodies/ligands that can recognize and selectively bind to antigens or receptors overexpressed on tumor cells can be more promising (Fig. 2). Also, the specificity and delivery efficiency of passively targeted nanosystems can be remarkably improved when tumor targeting ligands are also made part of the delivery systems [94]. One such nanoparticulate delivery system developed in our group is based on epidermal growth factor receptors (EGFR) peptide-grafted nanoparticles for active targeting to MDR ovarian cancer cells overexpressing EGFR [95, 96]. Werner *et al.* have used folate-based active targeting to deliver nanoparticles to ovarian cancer that overexpress folate receptors [97]. Such particles are internalized by active transport *via* receptor-mediated endocytosis [98]. In another example of active targeting, the arginine-glycine-aspartic acid (RGD) tripeptide was coupled to nanoparticles to target $\alpha_5\beta_5$ or $\alpha_5\beta_3$ integrin receptors that are overexpressed on vascular endothelial cells of angiogenic blood vessels of the proliferating tumor cells [99]. Nucleic acid construct such as aptamers that can selectively bind to prostate-specific membrane antigen (PSMA) on prostate cancer cells are also being pursued as active targeting agents to target prostate tumors [100].

In effect, active targeting using ligands or antigens coupled to nanoparticles serve as secondary targeting mechanism for binding/intracellular trafficking of the drug payload [101] after the primary (passive) targeting based on the EPR effect (Fig. 2). Such multimodal smart delivery systems are currently intensely pursued and have the potential to revolutionize cancer theranostics [102].

3.3. Multifunctional Polymeric Nanoparticulate Delivery Systems

The aim of developing a multi-functional nanoparticulate delivery system is to achieve several inter-related goals using a single “nanoplatfrom”. For instance, a nanosystem can be designed with low, moderate, or high level of complexity depending on the requirements of the delivery system and the disease target in the body such as: *i*) protection of the payload from degradation on systemic delivery and elimination of the side effects associated with free drug [103]; *ii*) evasion of the immune system and reduction in RES clearance by surface functionalization of nanoparticles with stealth molecules, such as PEG, thereby enabling long circulation half-life *in vivo* [104]; *iii*) to take advantage of the nano-size and passively target tumor tissues based on the EPR effect [105]; *iv*) to load with single (and often large doses of) or multiple therapeutic payloads that works synergistically to have a compounded

effect on tumor therapy [106, 107]. In this regard, biodegradable polymeric nanoparticles based delivery also offers the flexibility to devise the system to have spatial and temporal control (or controlled erosion characteristics) [108–110]. For example, the delivery vehicle can be engineered to release one agent concomitantly with another or if desired, after a time delay, or in a controlled fashion based on the therapeutic requirement of the payloads [108]; v) surface decoration with targeting molecules such as antibodies or peptides that can help locate, bind, and/or internalize the nanoparticles into the tumor cells, or with agents that can trigger the release of the payload (drugs/genes) in specific location or organelles within the tumor cells [89, 111–114]; and, vi) targeted and non-targeted nanoparticles can be designed to facilitate intracellular distribution of the payload that can be critical for many drugs and genes whose sites of action are generally located in the cytoplasm or the nucleus [115]. For example, plasmid DNA, oligonucleotides, micro RNA, *siRNA*, peptides, and proteins have their targets in the cytoplasm or nucleus. Thus, there is a need for these molecules to be transported inside the cell [115]. More importantly, intracellular trafficking of drugs and genes is more challenging for morbid and recalcitrant tumor cells equipped with drug efflux pumps such as P-glycoprotein (P-gp) and MDR proteins, which are responsible for MDR [116]. In such cases, nanoparticle-based delivery systems have shown to evade the drug efflux pathway, thus increasing the intracellular drug delivery efficiency [117, 118].

4. IMAGE-GUIDED DELIVERY SYSTEMS FOR CANCERS

4.1. Rationale for Image-Guided Delivery

The concept of image guidance as an integral component of drug delivery system has major advantages over conventional methodologies of drug treatment. For instance, in the conventional scenario an anticancer drug or a therapeutic agent is systemically administered to cancer patients. This type of delivery often lacks targeting of drugs/genes to specific organs/tissues or sites of interest (tumors), and there is neither any means of (*in situ*) tracking or imaging the *in vivo* fate nor the ability to measure the delivery efficiency of drugs/genes. Also, the bioavailability, therapeutic efficacy, and dose response of drug/gene treatment has to be estimated based on separate sets of experiments. For example, imaging the regression of tumors in patients after drug treatment by imaging modalities such as X-ray CT or radiographic examination will in itself be a separate intervention. In this regard, co-administering the image guiding molecules as part of the delivery system can help to achieve multiple goals in a single dosing, such as real-time and concurrent assessment of drug delivery efficiency/targeting, *in vivo* fate of drug and sites of localization/accumulation, modes of excretion, as well as imaging and monitoring the progress of drug treatment.

4.2. Role of Imaging in Nanotechnology

As discussed above, molecular imaging using conventional molecules such as contrast agents and optical/radiolabeled probes based on molecular markers have played a key role in disease identification, monitoring, and staging of cancers. Nanotechnology is another important area of science that is growing rapidly, which involves development of novel materials at the nanometer length dimensions, with unique physico-chemical properties that neither resemble the bulk nor the native molecular forms of the individual components [119]. Apart from catering to the broad interest in the area of biomedical research,

nanotechnology is poised to play a leading role in biomedical imaging especially for medical conditions such as cancer [120]. In this regard, the merging of nanotechnology with molecular imaging provides a versatile platform for novel (nano-) probe design that can augment the capabilities of conventional imaging agents such as enhanced sensitivity, specificity and signal amplification and also provide a modular platform for devising systems with multifunctional features. For instance, nanoparticles can be designed with fluorescent dyes, radioactive probes, contrast agents, and therapeutic molecules (anticancer drugs and oligonucleotides), all in a single construct that can facilitate multimodal and multipronged strategies for simultaneous detection, prognosis, and treatment of cancers. [59, 107, 121–124].

Many of the biocompatible polymers are suited to encapsulate drugs and therapeutic molecules in high concentrations/loading [125]. Furthermore, many polymeric systems have versatile (and often abundant) functional groups that can: *i*) facilitate synthesis of novel block copolymers that can assist in self assembly [125, 126]; or, *ii*) be used for conjugation of multiple types of imaging probes/drugs [127]; as well as, *iii*) targeting ligands for multimodal (targeted-) imaging [128]. Such multifunctional polymeric systems can form stable self-assembled nanostructures capable of drug/gene encapsulation, with built-in image guidance [129]. Nanoparticles are also of great value in evaluating the stability and *in vivo* fate of the delivery systems itself for developing robust nanosystems for future clinical applications. Examples of these nanosystems include dendrimers, liposomes (Fig. 3), polymeric micelles and nanoparticles [117, 130–137].

For the development of such diverse multifunctional image-guided delivery systems, traditional “one polymer-at-a-time” approaches are laborious and time consuming and require large amount of resources for optimization and arriving at the right formulation for the intended therapeutic application. In this respect, combinatorial approaches and high throughput polymer synthesis and screening are needed [138–140]. Also, high-throughput methods can be used for better understanding of structure-property relationship between the components forming the nanosystems such as carrier polymer and the drug/genes encapsulated within them. In our laboratory, we are developing a novel “mix and match” type combinatorial polymer library screening [139] and depending on the type of biodegradable/biocompatible carrier polymers, their molecular weight, surface charge, functionality, and hydrophilic/hydrophobic character they can be matched with the drug or gene of interest [139]. In this regard, targeting the polymeric nanosystems with ligands (biomarkers, antibodies, peptides), and imaging (MRI, optical, radioactive) probes can provide a quick and reliable assessment on the right ratios of the building blocks for efficient and stable encapsulation of drugs and genes into the nanoparticles [71]. This type of approach will, therefore, expedite the rational design and development of multifunctional nanosystems in cancer therapy. Another important criterion in selection of materials is the inherent toxicity/non-compatibility of some of the components used in building the multi-component delivery systems. The combinatorial “mix and match” type screening can thus be useful in developing and identifying nanosystems that can be designed, for instance to be biocompatible or devoid of immunogenicity/toxicity or complement activation *in vivo*, by the right choice/selection of materials such as incorporation of counter ion containing moieties to reduce surface charge or by including PEG-modified blocks to prevent immune

recognition [141]. The schematic representation of formation or self-assembly of multifunctional nanoparticles from the corresponding building blocks is shown in Fig. (4).

5. ILLUSTRATIVE EXAMPLES OF IMAGE-GUIDED DRUG DELIVERY SYSTEMS FOR CANCER

The approach of using imaging and contrast agents for monitoring drug delivery has already been a part of clinical intervention. For example the first prototype polymer conjugated drug SMANCS dissolved in a iodinated lipid contrast medium (Lipiodol®) is used clinically for simultaneous drug delivery, tumor detection, and measuring the delivery efficiency in patients using X-ray CT [142].

Recently, more sophisticated and complex designs for image guided drug delivery are being assessed in the *in vitro* and preclinical settings by utilizing nanotechnology. Along these lines, Yu *et al*, have developed PSMA aptamer-conjugated to superparamagnetic iron oxide nanoparticles loaded with doxorubicin that could be used for prostate cancer-specific nanotheranostics [143]. These agents are capable of not only detecting prostate tumors *in vivo* (by MRI) but also can selectively deliver doxorubicin to the tumor tissues [143].

In another study, Santra *et al*. have used multimodal optical and MRI agents containing biocompatible nanoparticles that could demonstrate targeted optical/MR imaging and cell killing towards folate receptors expressing cancer cells [144]. A modified solvent diffusion method was also developed for co-encapsulation of both an anticancer drug (docetaxel) and NIR dyes into nanoparticles, which are new additions to the arsenal of nano-delivery systems for detection, diagnosis, and treatment of cancers [144].

Ross's group has been actively pursuing light-activable theranostic nanoparticles for the imaging and photodynamic therapy of brain tumors [145]. In this construct, both iron oxide nanoparticles and photofrin (a potent photosensitizer) was incorporated within PEG-modified polyacrylamide nanoparticles [145]. Further, the particles were tagged with a vascular homing (F3) peptide that binds selectively to nucleolin on angiogenic endothelial cells and tumor cell surfaces. These particles demonstrated selective targeting and pronounced antitumor effect in rat brain animal tumor models [145].

In another approach, quantum dot (QD)-aptamer conjugates were used for concurrent cancer imaging and therapy of doxorubicin delivery based on the bi-fluorescence resonance energy transfer (Bi-FRET) technique [146]. The surface of quantum dots was functionalized with a RNA aptamer that could recognize the extracellular domains of PSMA. The anticancer drug, doxorubicin was intelligently loaded onto the nanosystem by intercalation of drug in the double-stranded stem of aptamer forming the QD-aptamer (doxorubicin) conjugate with reversible self-quenching properties based on a Bi-FRET mechanism [146]. This smart multifunctional nanoparticulate system was able to deliver doxorubicin to the targeted prostate cancer cells as well as sense and image the drug delivery to the cancer cells by activating the fluorescence of QDs [146]. Such advanced delivery systems demonstrate the versatility of nanoparticles for highly specific, sensitive and therapeutically effective strategies for simultaneous disease detection and therapy.

Koo *et al.* have used pH responsive polymeric micelles for simultaneous non-invasive *in vivo* imaging and photodynamic therapy of tumors in mice models [147]. A Michael type addition reaction was utilized to conjugate a pH-sensitive polymer, poly(*b*-amino ester) with methoxyPEG. The block copolymer could self assemble with hydrophobic radiosensitizer protoporphyrin IX (PpIX), forming stable nanosized micelles. These micelles showed marked pH-responsive demicellization and release of the photosensitizer in the tumors due to the acidic pH conditions. Furthermore, the micelles showed clear tumor accumulation as assessed by fluorescence imaging and complete tumor ablation when irradiated with laser light in tumor bearing mice models, thereby demonstrating their potentials for photodynamic theranostics [147].

6. ILLUSTRATIVE EXAMPLES OF IMAGE-GUIDED NUCLEIC ACID DELIVERY FOR CANCER

Gene therapy based on RNA interference (RNAi) mechanism has been well established and has currently become a major area of research that hold great promise for the management of diseases such as cancers. Although RNAi agents have high efficiency and specificity, the major hurdle still remains its delivery to target tumor/tissues and intracellular trafficking after localization in the sites of interest [148]. In this regard, the ability to image/detect and deliver *sRNA* has become increasingly important. Researchers in Moore's group have developed a dual-purpose probe for *in vivo* delivery and simultaneous imaging of *sRNA* accumulation in tumors by using high-resolution MRI and NIR *in vivo* optical imaging techniques [149]. The *sRNA* was covalently conjugated to nanoparticles consisting of NIR dye conjugated magnetic nanoparticles. Furthermore, the nanoparticles were decorated with a specific membrane translocation peptide that enables their intracellular delivery and cytosolic availability [149]. Taken together, their study demonstrates the feasibility of *in vivo* tracking of *sRNA*, *in vivo* dual modality imaging of tumor uptake of nanoparticles, as well as silencing ability of the *sRNA*-conjugated nanoparticles [149].

In another study, Kumar *et al.* used superparamagnetic iron oxide nanoparticles as a construct for combined optical/MR imaging and delivery of *sRNA* to solid tumors [150]. The nanoconstruct, apart from performing its function as a dual modality imaging agent and a vehicle for the delivery of *sRNA* also served as a tool to study basic tumor biology and therapy [150]. The nanoconstruct consisted of magnetic nanoparticles (for MRI), a Cy3 fluorophore (for optical imaging), a targeting peptide (EPPT) that could home to a specific antigen (uMUC-1) overexpressed by the majority of human breast cancer cells, and a synthetic *sRNA* that downregulates the *BIRC5* antiapoptotic gene in breast cancer cells [150]. The nanoconstruct demonstrated specific tumor uptake and significant downregulation of *BIRC5* gene, in animal tumor models. More importantly, the tumor uptake could be visualized both by MRI and NIR optical imaging [150].

Researchers from Park's group developed a polyelectrolyte complex micelle-based VEGF *sRNA* delivery system for anti-angiogenic gene therapy [141]. The complexation of the PEG-conjugated VEGF *sRNA* was achieved by charge interaction with poly(ethyleneimine) (PEI). This complex formed a stable core-shell nanostructure with the PEI/*sRNA* forming

the central core and PEG chains forming the corona. The i.v. and i.t. injection of the micelles in mice demonstrated significant inhibition of VEGF expression in the tumor tissue and suppressed tumor growth without detectable toxicities [141]. Furthermore, to confirm the feasibility of *siRNA*-based gene therapy and imaging *in vivo*, optical imaging was undertaken using a Cy5.5 labeled *siRNA*. The Cy5.5 labeled *siRNA* containing polyelectrolyte complex micelles predominantly accumulated in the tumor, affirming the feasibility of this approach [141].

In order to overcome the dose-limiting side effects of conventional chemotherapeutic agents and the therapeutic failure due to MDR, we designed and evaluated a novel biocompatible lipid-modified dextran-based self-assembled polymeric nanosystem that could encapsulate *MDR-1 siRNA* as well as doxorubicin [71, 151]. Further, in order to image the transfection efficacy of *siRNA*-loaded nanoparticles on cell lines we utilized the green fluorescence protein (GFP) expressing BHK-21 cells, and performed confocal fluorescence microscopy to visualize the downregulation of GFP. It was observed the GFP *siRNA* was efficiently incorporated into cells and effectively inhibited the expression of GFP in a dose dependent manner. Similarly, the MDR1 *siRNA*-loaded dextran nanoparticles efficiently suppress P-gp expression in a drug resistant osteosarcoma cell lines. A combination therapy of the MDR1 *siRNA*-loaded nanocarriers with doxorubicin revealed pronounced increase in drug uptake in the nucleus (assessed by confocal fluorescence microscopy imaging) of MDR cells. These results demonstrate that our approach may be useful in reversing MDR by increasing the amount of drug accumulation in MDR cells. Thus, the delivery using non-toxic and biocompatible dextran-based nanosystems offers a versatile platform for the incorporation of multiple payloads for simultaneous imaging and delivery of therapeutic molecules that can be translatable for human clinical applications.

In another study, Huh *et al.* designed a *siRNA* delivery system containing the biodegradable and biocompatible polymer glycol chitosan (GC) and PEI (Fig. 5) [152]. The polymers were conjugated with 5 β -cholanic acid (CA) to stabilize the nanoparticles and endow them with tumor-homing ability. The nanoparticles were formed by mixing GC-CA and PEI-CA to form self-assembled GC-PEI nanostructures, due to the strong hydrophobic interactions of 5 β -cholanic acids in the polymers. The cationic charge on the GC-PEI nanoparticles was used to complex with the negatively charged red fluorescence protein (RFP) gene silencing *siRNA* designed to inhibit RFP expression. *In vitro* studies with RFP expressing B16F10 tumor cells incubated with *siRNA*-GC-PEI nanoparticles revealed time-dependent cellular uptake of the nanoparticles, and lead to a significant inhibition of RFP gene expression in RFP/B16F10-bearing mice models, thus demonstrating their potentials as a promising vector for *siRNA* delivery (Fig. 5) [152].

CONCLUSIONS

Multifunctional nanoparticles utilizing the active and passive tumor targeting principles has played a leading role in addressing some of the critical issues related to cancer drug delivery, such as overcoming the inherent limitations of drug/genes (with regard to stability, non-specificity, and short half-life), and the anatomical and patho-physiological barriers in delivering them specifically to tumor tissues. In this respect, the role of image guidance is of

paramount importance in drug delivery and many of the nanosystems currently employ image guiding molecules or drugs/genes labeled with imaging probes for several reasons, i.e., measuring delivery efficiency, selectivity, sites of localization of drugs/genes, and concurrent measurement of the drug treatment response (such as reduction in tumor volume). Also, *in situ* imaging of the molecular events while the drug is being delivered using nanoparticles can provide startling insights about the molecular mechanism of tumor progression (such as its invasion and metastatic behavior) that can assist a great deal in the future design and development of novel molecular nanoprobe-based delivery systems. More importantly, the development of such image-guided delivery systems can aid in the early detection of cancers, which can be regarded as one of the most powerful tools in deciphering possible cures for this deadly disease. These types of advanced multifunctional precision image-guided nanosystems are thus envisaged to play a major role in the era of personalized cancer therapy.

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ABBREVIATIONS

Bi-FRET	bi-fluorescence resonance energy transfer
BK	bradykinin
CA	5 β -cholanic acid
CT	X-ray computed tomography
EGFR	epidermal growth factor receptors
EPR	enhanced permeability and retention
¹⁸F-FDG	2-[¹⁸ F]-fluoro-2-deoxy-D-glucose
FMT	3D-fluorescence molecular tomography
FRI	2D-fluorescence reflectance imaging
GC	glycol chitosan
GFP	green fluorescence protein
i.a.	intraarterial
i.t.	intratumoral
i.v.	intravenous
MDR	multi-drug resistance
MMPs	matrix metalloproteinases

MRI	magnetic resonance imaging
NIR	near-infrared
NO	nitric oxide
PEG	poly(ethylene glycol)
PEI	poly(ethyleneimine)
PET	positron emission tomography
P-gp	P-glycoprotein
PGs	prostaglandins
PpIX	protoporphyrin IX
PSMA	prostate-specific membrane antigen
QD	quantum dot
RES	reticuloendothelial system
RFP	red fluorescence protein
RGD	arginine-glycine-aspartic acid
RNAi	RNA interference
siRNA	small interfering RNA
SPECT	single photon emission computed tomography
VEGF	vascular endothelial growth factor

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Fig. (1).

Novel radiolabeled scFv antibodies for targeted imaging of tumors. The figure shows *in vivo* tumor targeting and imaging of ^{99m}Tc -labeled M40 single chain fragment antibody (scFv) in mouse bearing both sarcomatoid (VAMT-1) and epithelioid (M28) mesothelioma. (A) Single photon emission computed tomography/X-ray computed tomography (SPECT/CT) fused coronal image of ^{99m}Tc -M40 imaged 3 hours after injection. (B) 3D fused SPECT/CT coronal image. (C) blocking control study (the target-mediated uptake is confirmed by $\approx 70\%$ reduction in tumor activity following administration of 10-fold excess of unlabeled scFv). (D) Positron emission tomography/X-ray computed tomography (PET/CT) fused coronal image of 2- ^{18}F -fluoro-2-deoxy-D-glucose (^{18}F -FDG) imaged 1 hour after injection (for comparison). The figure shows the ability of the novel M40 scFv to target both sarcomatoid (VAMT-1) and epithelioid (M-28) mesothelioma tumors effectively in mouse models demonstrating the utility of such agents in imaging/detection of all subtypes of mesotheliomas. Adapted with permission from Ref. [29]. Copyright AACR (2011).

Fig. (2).

Passive and active tumor drug targeting. The scheme shows the passive and active targeting mechanisms of multifunctional image guided nanoparticles and the difference in the vasculature of normal and tumor tissues. Drugs and small molecules diffuse freely in and out of the normal and tumor blood vessels due to their small size and thus the effective drug concentration in the tumor drops rapidly with time. On the opposite, macromolecular drugs and nanoparticles can passively target tumors due to the leaky vasculature, but they cannot diffuse back into blood stream due to their large size (EPR effect). Targeting molecules such as antibodies or peptides present on the nanoparticles can selectively bind to cell surface receptors/antigens overexpressed by tumor cells and can be taken up by receptor-mediated endocytosis (active targeting). The image guiding molecules and contrast agents conjugated/encapsulated in the nanoparticles can be useful for targeted imaging and (non-invasive) visualization of nanoparticle accumulation/localization, as well as for mechanistic understanding of events and efficacy of drug treatment simultaneously.

Fig. (3). Radiolabeled immunoliposomes for targeted imaging of tumors. SPECT/CT fused images (coronal view, and transverse view) of ^{111}In -labeled targeted immunoliposomes taken after 24 h after injection in mouse bearing both sarcomatoid (VAMT-1) and epithelioid (M28) mesothelioma. The uptake of the radiolabeled immunoliposome in both epithelioid (M28) and sarcomatoid (VAMT-1) subtypes of mesothelioma tumors at 24 h is clearly seen. Adapted with permission from Ref. [101]. Copyright Elsevier (2011).

Fig. (4). Multifunctional nanosystems for theranostics. Schematic representation of the formation of: (A) multifunctional self-assembled targeted nanoparticle, and (B) multifunctional targeted radio-immunoliposome from the corresponding building blocks. The multifunctional nanoparticles and liposomes can be engineered by self-assembly of polymers/lipids containing various functionalities such as amphiphilic block copolymers, to facilitate formation of stable nanoparticles loaded with drugs, genes, and imaging agents; antibody and radiolabeled polymer blocks for targeting and imaging; as well as, PEG chains for enabling long circulation half-life *in vivo*.

Fig. (5).

Image-guided gene silencing using polymeric nanoparticles. (A) Schematic representation of *siRNA*-loaded in hydrophobically-modified GC and PEI nanoparticle (*siRNA*-GC-PEI NP). (B) *In vivo* NIR fluorescence imaging of SCC7 tumor-bearing mice 1 h post-injection of Cy5.5-*siRNA*-GC-PEI NP. (C) *In vivo* knockdown of targeted proteins by *siRNA* (left image) and *siRNA*-GC-PEI NP (right image) after i.v. injection in B16F10-RFP tumor-bearing mice. The mice were observed by dual modality imaging using light microscopy and by NIR fluorescence imaging system. Adapted with permission from Ref. [152]. Copyright Elsevier (2010).