Nanooncology: The Future of Cancer Diagnosis and Therapy

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In recent years, there has been an unprecedented expansion in the field of nanomedicine with the development of new nanoparticles for the diagnosis and treatment of cancer. Nanoparticles have unique biological properties given their small size and large surface area-to-volume ratio, which allows them to bind, absorb, and carry compounds such as small molecule drugs, DNA, RNA, proteins, and probes with high efficiency. Their tunable size, shape, and surface characteristics also enable them to have high stability, high carrier capacity, the ability to incorporate both hydrophilic and hydrophobic substances and compatibility with different administration routes, thereby making them highly attractive in many aspects of oncology. This review article will discuss how nanoparticles are able to function as carriers for chemotherapeutic drugs to increase their therapeutic index; how they can function as therapeutic agents in photodynamic, gene, and thermal therapy; and how nanoparticles can be used as molecular imaging agents to detect and monitor cancer progression. **CA Cancer J Clin 2013;63:395-418.** **©2013 American Cancer Society, Inc.**

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Introduction

Current treatment options for cancer include a combination of surgery, radiation therapy, and chemotherapy. Over the past decade, our ability to design new treatments for cancer has been facilitated by a greater understanding of the tumor microenvironment. Cancer tissue is composed of noncellular (ie, vascular and interstitial) and cellular compartments that differ remarkably compared with the surrounding normal tissue. Each of these compartments provides a challenge for the local delivery of drugs to tumor cells (Fig. 1).

Within the noncellular compartment, tumor vascularity is markedly heterogeneous with densely vascularized areas supplying oxygen and nutrients to rapidly growing parts of the tumor while regions of tumor necrosis, in contrast, receive little blood supply. In addition, there is a decreasing amount of oxygen available to tumor cells which are further away from blood vessels; this is due in part to the increased distance over which oxygen has to diffuse to reach these cells as well as the consumption of oxygen by tumor cells that are closer to the blood vessels. New blood vessels are synthesized by tumors in a process known as angiogenesis; however, these vessels are abnormal with increased numbers of proliferating endothelial cells, increased vessel tortuosity, deficient pericytes, and abnormalities in the basement membrane with large gaps between adjacent endothelial cells ranging between 380 and 780 nanometers (nm).[1,2] In addition, vascular endothelial growth factor, bradykinin, prostaglandins, and nitric oxide are all upregulated and contribute to the hyperpermeable nature of tumors. Surrounding the tumor cells is the interstitial environment, which is composed of a collagen and elastic fiber network.[3] Unlike normal tissues, the tumor interstitium has high interstitial pressure and a relative absence of a functioning lymphatic network. The combined effect of a "leaky" defective vascular architecture and poor tumor lymphatic drainage is responsible for the enhanced permeability and retention (EPR) effect.[4,5] Although the EPR effect helps to deliver chemotherapeutic agents to well-vascularized parts of the tumor, drugs may not reach the poorly vascularized regions, thereby preventing some cancer cells from receiving cytotoxic treatment. This effect is further compounded by low microvascular pressure in

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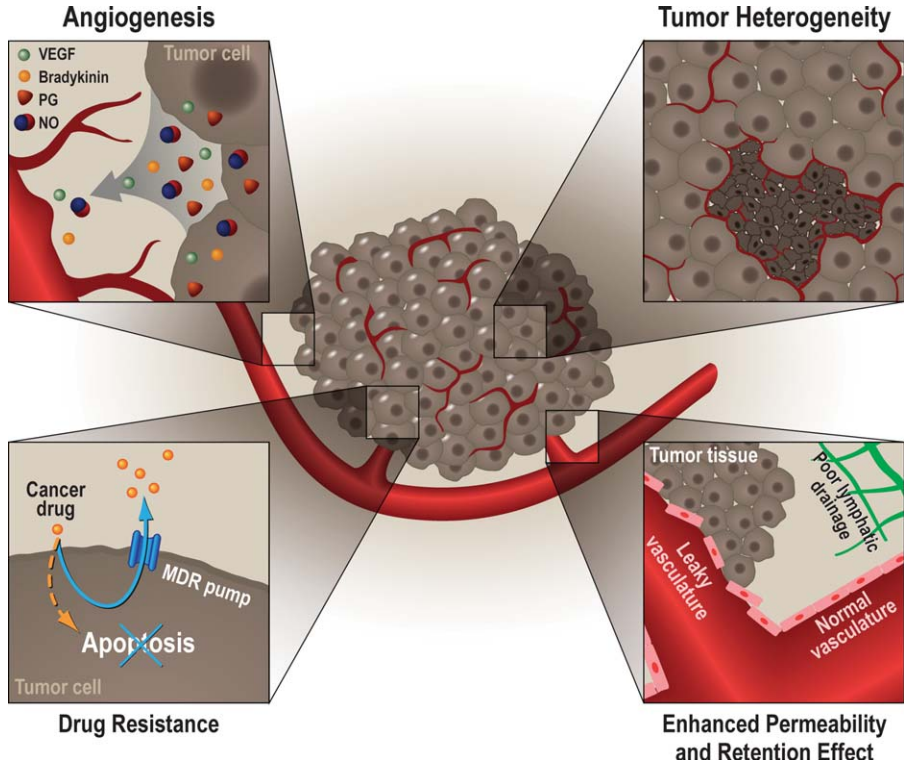

FIGURE 1. The Tumor Microenvironment. (Top left insert) Angiogenesis due to tumor cells releasing factors (ie, vascular endothelial growth factor [VEGF], bradykinin, prostaglandins [PGs], and nitric oxide [NO]) that stimulate the production of new blood vessels. (Top right insert) Tumor heterogeneity as demonstrated by areas of tumor necrosis surrounded by viable tumor cells and areas of good and poor tumor perfusion. (Bottom left insert) An example of drug resistance in tumor cells due to the upregulation of multidrug resistance-associated protein pumps (MDRs), which are capable of extruding chemotherapeutic drugs out of the cell. (Bottom right insert) The enhanced permeability and retention due to the combined effect of "leaky" defective vascular architecture and poor tumor lymphatic drainage. This allows chemotherapeutic drugs to leave the vasculature and accumulate at the site of the tumor.

these regions, which reduces the extravasation of drugs from the vasculature given the surrounding high interstitial pressure. In addition, the reduction of available oxygen due to the lack of vasculature results in an acidic microenvironment from the buildup of lactic acid via anaerobic glycolysis, which, in turn, confers resistance against basic drugs that are ionized thereby preventing their diffusion across the cell membrane. Taken together, these factors likely account for the noncellular mechanisms of drug resistance.

Recent studies have now shown that there are 2 distinct populations of cells within a tumor: a small rare and quiescent population known as cancer stem cells, and another larger population of rapidly proliferating cells that forms the majority of the tumor mass. While noncancer stem cells do not have the capacity to self-sustain or metastasize, cancer stem cells not only have the ability to regenerate the tumor but also retain their genetic programs for cell migration (ie, invasion and metastasis) and self-protection. Because most therapeutic treatments mainly target noncancer stem cells, this leaves the cancer stem cells behind, which can then regenerate the tumor explaining why tumors often recur after treatment. Hence, new treatments are being designed to specifically target cancer stem cells, which are now believed to be the critical therapeutic target. The destruction of these cells will permanently eradicate cancer cells, thereby preventing local recurrence and metastasis.[6] In addition, the microenvironment around the cancer stem cells has also been shown to provide signals to control their proliferation and other cell-fate decisions, thereby enabling tumors to express their full neoplastic phenotype. Hence, strategies to manipulate the nonmalignant cells in the microenvironment may provide another option for treating and/or controlling the evolution and neoplastic nature of cancer. One such target is tumor-associated macrophages (TAMs), which are recruited to tumors by growth factors and chemokines (ie, colony-stimulating factor-1) produced by the tumor cells.[7] TAMs are found in abundance in the stroma of solid tumors and have been shown to enhance tumor progression by promoting tumor invasion, migration, and angiogenesis.[8] This is supported by the good correlation noted between high TAM infiltration and poor patient outcome.[9]

Inside cancer cells, there are biochemical and metabolic changes resulting in alterations in enzyme activity, apoptosis regulation, and intracellular and extracellular transport

mechanisms, all of which contribute to the cellular mechanisms of drug resistance. Perhaps the most significant example is the upregulation of the multidrug resistance-associated protein pumps, also known as P-glycoprotein, which is an ATP-binding cassette transporter that is capable of extruding several chemotherapeutic drugs across the plasma membrane and out of the cell, thereby reducing the drug-target interaction. Furthermore, the full therapeutic benefit of many chemotherapeutic drugs is limited by their nonspecific systemic biodistribution, which results in systemic cytotoxicity and lower concentrations of drug delivered directly to the tumor.[10]

Current research has therefore focused on developing more efficient local drug delivery or drug-targeted therapies to overcome these obstacles. New therapies are being designed to deliver chemotherapeutic drugs to the tumor at higher concentrations with minimal damage to normal tissues. Examples include drugs conjugated with monoclonal antibodies that bind to molecular targets that are solely expressed on cancerous cells. This allows the drug to be specifically directed to the tumor while limiting its exposure to normal cells that do not significantly bind with the attached antibody. Nevertheless, studies have shown that only 1 to 10 parts per 100,000 of intravenously administered monoclonal antibodies reach their parenchymal targets in vivo, with similar limitations noted for molecular imaging agents.[10-12] A new emerging strategy to overcome these problems is to use nanoparticles for drug delivery, tumor therapy, and tumor follow-up using different imaging modalities.

In recent years, there has been an unprecedented expansion in the field of nanomedicine, with the development of new nanoparticles for the diagnosis and treatment of diseases such as cancer. Nanoparticles have unique biological properties given their small size, allowing them to have a surface area-to-volume ratio that is larger than that of other particles. Their large functional surface area allows them to bind, absorb, and carry other compounds such as small molecule drugs, DNA, RNA, proteins, and probes. Furthermore, their tunable size, shape, and surface characteristics enable them to have high stability, high carrier capacity, the ability to incorporate both hydrophilic and hydrophobic substances, and compatibility with different administration routes, thereby making them highly attractive in many aspects of medicine. Although the design (ie, shape and size) and material from which nanoparticles are made will ultimately determine their physicochemical properties, nanoparticles in general are relatively stable over large ranges of temperature and pH. However, the lack of biodegradation and slow dissolution rates of some nanoparticles raises concern over their safety, especially for long-term administration. Nanoparticles can be categorized into those made from biological-like materials (ie, phospholipids, lipids, dextran, and chitosan), carbon-based materials (ie, carbon nanotubes), and inorganic nanoparticles (ie, those based on metals, metal oxides, and metal sulfides), which also include semiconductor nanoparticles (ie, quantum dots [QDs]). Depending on the composition, their interaction with cells will be quite different.

This review article will discuss how nanoparticles are able to function as carriers for chemotherapeutic drugs to increase their therapeutic index; how they can function as therapeutic agents in photodynamic, gene, and thermal therapy; and how nanoparticles can be used as molecular imaging agents to detect and monitor cancer progression.

Nanoparticles as Carriers for Drug Delivery

Drug delivery is one of the major areas in which nanotechnology is helping revolutionize the treatment of cancer. Nanoscale complexes currently being developed consist of 2 main components: the nanoparticle itself, which is used as the carrier agent, and the chemotherapeutic drug.[11] The drug can either be adsorbed, dissolved, or dispersed throughout the nanoparticle complex or, alternatively, it can be covalently attached to the surface. In addition to engineering nanoparticles for drug delivery, chemotherapeutic drugs themselves can also be formulated at a nanoscale level. Studies using paclitaxel have shown that when compared with the conventional formulation, the nanoparticle formulation of the drug increases both its cytotoxicity profile in cell culture and its therapeutic efficiency in a living animal model.[13] This has been attributed to the nanoparticle formulation having greater bioavailability and a longer sustainable therapeutic time, which allows the drug concentration to remain above the minimum effective value for an extended period of time. In addition, the nanoparticle formulation overcomes issues associated with the current formulation of paclitaxel, which includes low water solubility and severe side effects associated with the adjuvant Cremophor EL.

For nanoparticle-drug complexes to be effective in delivering their payloads directly to cancer cells in living subjects, they must fulfill certain criteria (Fig. 2):

- The nanoparticle must be able to bind or contain the desired drug(s).
- The nanoparticle-drug complex must remain stable in the serum to allow systemic delivery of the drug.
- The nanoparticle-drug complex has to be delivered to tumor cells (either by receptor-mediated interactions or via the EPR effect), thereby reducing any unwanted complications from nontargeted delivery.
- The nanoparticle must be able to release the drug once at the site of the tumor.
- The residual nanoparticle carrier should ideally be made of a biological or biologically inert material with a limited lifespan to allow safe degradation.

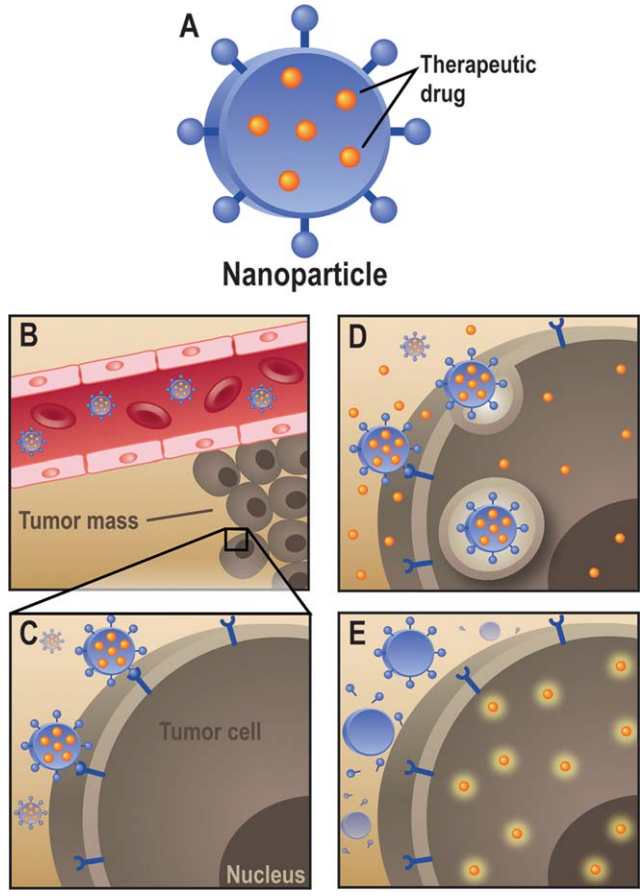

FIGURE 2. The Criteria Nanoparticles Need to Fulfill to Be Effective Carriers for Chemotherapeutic Drugs. (A) The nanoparticle carrier must bind or contain the desired chemotherapeutic drug(s). (B) The nanoparticle-drug complex must remain stable in the serum to allow for the systemic delivery of the drug. (C) The nanoparticle-drug complex must be delivered only to tumor cells. (D) The nanoparticle must be able to release the drug once at the site of the tumor. (E) After drug delivery, the residual nanoparticle carrier must be safely degraded.

Alternatively, if a nonbiodegradable material is used, it must be proven to be safe at the doses needed or clear from the subject.

The Nanoparticle-Drug Complex

Nanoparticles that are used as carriers will either bind the drug on their surface or entrap and encapsulate the drug to protect it from degradation or denaturation. Nanoparticle carriers also offer the potential to codeliver 2 or more drugs simultaneously for combination therapy. Newer applications also include the delivery of noncytotoxic prodrugs that can be activated once they are delivered to cancer cells (ie, platinum [Pt]-based chemotherapeutic agents can be photoreduced using visible light from their Pt [IV] prodrug state to the active Pt [II] anticancer drug once delivered inside cells using nanoparticle carriers).[14] There are several types of nanoparticle systems that have been used as carriers including liposomal, solid lipid, polymeric, mesoporous silica, and inorganic nanoparticles.

Liposomes are a biologically based nanoparticle system made from a self-assembling concentric lipid bilayer that is primarily composed of amphipathic phospholipids enclosing an interior aqueous space. They are able to contain hydrophilic drugs, which can remain encapsulated in the central aqueous interior, and can be designed to adhere to cell membranes and release drugs after endocytosis. Studies have shown improved pharmacokinetics and pharmacodynamics of drugs associated with liposomes.[15] Over the years, liposomes have been surface modified with glycolipids and/or polyethylene glycol (PEG) to prevent their rapid clearance from the circulation system by mononuclear phagocytic cells from the reticuloendothelial system (RES).[16] The addition of PEG or other hydrophilic conjugates to the surface of all types of nanoparticle carriers, including liposomes, provides increased stability of the nanoparticle in biological fluids while also creating a dynamic cloud of hydrophilic and neutral chains at the surface that reduces protein opsonization thereby enabling nanoparticles to partially evade the macrophages of the RES.[2] This will increase nanoparticle half-life in blood, which combined with their ability to conjugate targeting moieties, will allow them to preferentially accumulate at

TABLE 1. Examples of Nanoparticles Used in Cancer Therapy

TRADE NAME	DESCRIPTION OF NANOPARTICLE	CANCER TARGETED BY THE NANOPARTICLE	PHASE OF DEVELOPMENT
Abraxane	Albumin-bound paclitaxel	Metastatic breast cancer[24]	Approved
Doxil	Liposomal doxorubicin	HIV-related Kaposi sarcoma, metastatic breast and ovarian cancer[25]	Approved
DaunoXome	Liposomal daunorubicin	HIV-related Kaposi sarcoma[26,27]	Approved
Myocet	Liposomal doxorubicin	EGFR2-positive metastatic breast cancer[28]	Approved
DepoCyt	Liposomal cytarabine	Intrathecal lymphomatous meningitis[29]	Approved
Marqibo	Liposomal vincristine sulphate	Acute lymphoblastic leukemia[30,31]	Approved
Oncaspar	Polymeric PEG-L-asparaginase	Acute lymphoblastic leukemia[32]	Approved
Zinostatin stimalamer	Copolymer styrene maleic acid-conjugated neocarzinostatin	Unresectable hepatocellular carcinoma[33,34]	Approved
Resovist	Carboxydextran-coated SPIO	MRI contrast agent for imaging hepatocellular carcinoma[35]	Approved
Genexol-PM	Polymeric methoxy-PEG-poly(D,L-lactide) paclitaxel	Metastatic breast cancer[36]	Approved
NanoTherm	Aminosilane-coated SPIO	Local ablation of glioblastoma multiform[37,38]	Approved
Xyotax	Poly-L-glutamic acid (poliglumex) conjugate with paclitaxel	Ovarian cancer and NSCLC[39]	Phase 3
NKTR-102	PEG micelle with irinotecan	Breast and colorectal cancer[40]	Phase 3
Mepact	Liposomal muramyl tripeptide phosphatidyl ethanolamine	Nonmetastatic resectable osteosarcoma[41]	Phase 3
ThermoDox	Liposomal nanoparticle with thermal release of doxorubicin	Hepatocellular carcinoma[42]	Phase 3
CRLX-101	Cyclodextrin-PEG micelle with camptothecin	Lung and ovarian cancer[43]	Phase 2
NKTR-102	PEG micelle with irinotecan	Ovarian cancer[40]	Phase 2
Genexol-PM	Polymeric methoxy-PEG-poly(D,L-lactide) paclitaxel	Non-small cell lung, pancreatic, bladder and ovarian cancer[36,44-46]	Phase 2
CRLX-101	Cyclodextrin-PEG micelle with camptothecin	Renal cell carcinoma[47]	Phase 1
Docetaxel-PNP	Polymeric nanoparticle formulation of docetaxel	Advances solid malignancies[48]	Phase 1
NanoTherm	Aminosilane-coated SPIO	Pancreatic and prostate cancer[37,49]	Phase 1
Cyclosert (CALAA-01)	siRNA targeting M2 subunit of ribonucleotide reductase in a β-cyclodextrin-PEG nanoparticle	Solid tumors[50,51]	Phase 1
SGT53-01	Transferrin-targeted liposome loaded with the p53 gene	Solid tumors[52,53]	Phase 1
MCC-465	Human antibody fragment-targeted liposomal doxorubicin	Metastatic stomach cancer[54]	Phase 1
Aurimmune	Gold nanoparticle loaded with tumor necrosis factor	Solid tumors[55]	Phase 1
AuroShell	Near-infrared irradiation with gold nanoshells (localized thermal ablation)	Head and neck cancers[56,57]	Phase 0 (pilot study)
C-dots	PEG-coated SiO_2	Melanoma[58]	IND approved

HIV indicates human immunodeficiency virus; EGFR2, epidermal growth factor receptor 2; PEG, polyethylene glycol; SPIO, superparamagnetic iron oxide; MRI, magnetic resonance imaging; NSCLC, non-small cell lung cancer; PNP, polymeric nanoparticle; siRNA, small interfering RNA; IND, Investigational New Drug.

the site of the tumor. Although it was initially believed that liposomes entered cells via fusion of their phospholipid membrane with the cell membrane, it is now believed that this process is due to endocytosis.[17] Current clinical trials are investigating the therapeutic benefits of using liposomes loaded with chemotherapy drugs, such as doxorubicin and daunorubicin, for the treatment of patients with solid tumors and hematological malignancies.[18-21] Doxil is a PEGylated liposome loaded with doxorubicin that has been shown to have improved pharmacokinetics and

reduced side effects when compared with its parent drug doxorubicin alone,[22] and has been approved by the US Food and Drug Administration (FDA) for the treatment of patients with ovarian and metastatic breast cancers and human immunodeficiency virus-related Kaposi sarcoma.[23] Some examples of nanoparticles used to treat cancer that have either been approved or are still in clinical development are shown in Table 1.[24-60]

Solid lipid nanoparticles (SLNs) were developed in the 1990s as an alternative carrier system to emulsions, liposomes, and polymeric nanoparticles. They are more stable than liposomes in biological systems due to their rigid core of hydrophobic lipids surrounded by a monolayer of phospholipids. In addition, these carrier systems combine the advantage of colloidal lipid emulsions with those of particles which have a solid matrix.[61] Because they are also biodegradable, they are less toxic than polymeric or mesoporous silica nanoparticles. SLNs are composed of 0.1% to 30% solid (weight/weight) lipid dispersed in an aqueous medium and, if necessary, stabilized with 0.5% to 5% (weight/weight) surfactant.[62] Because the parameters involved in SLN synthesis can be relatively easy to manipulate, SLNs can be engineered either with 1) a drug-enriched shell, 2) a drug-enriched core, or 3) as a homogenous matrix,[63,64] with each variant having a different drug release profile. Furthermore, because drugs have also been shown to enter the core of SLNs at lower temperatures and to exit at higher temperatures, techniques to induce hypothermia and hyperthermia can be used to load and unload SLNs with therapeutic agents. Because incorporated drugs are located between fatty acid chains, between the lipid layers and within crystal imperfections, a highly ordered crystal lattice cannot accommodate large amounts of drug. Therefore, by using more complex lipids (ie, monoglycerides, diglycerides, triglycerides, or different chain lengths), higher drug loading is made possible.[65] Nevertheless, the drug-loading capacity of conventional SLNs is limited at approximately 25% with regard to the lipid matrix. Changes in temperature during either storage or administration can also lead to polymorphic transitions, which may cause the premature expulsion of the drug from the lipid matrix. Derivatives of SLNs, referred in the literature as nanostructured lipid carriers and the lipid drug conjugates nanoparticles, have also been developed to overcome these limitations by increasing the drug payload and preventing drug expulsion.

A polymeric nanoparticle is a collective term given to any type of polymer nanoparticle, but specifically nanospheres and nanocapsules. Although nanospheres are generally spherical and solid with molecules attached or adsorbed to their surface, nanocapsules are vesicular systems with substances confined within a cavity consisting of a liquid core (either water or oil) surrounded by a solid shell.[66] Polymeric nanoparticles can be prepared from preformed polymers or by direct polymerization of monomers using classical polymerization or polyreactions.[67] The chemistry involved in their synthesis and preparation can be easily manipulated to allow desired properties to be built into the nanoparticle, such as surface modifications to improve biodistribution properties, pharmacokinetic control, and entrapment of therapeutic agents.[63] Studies have shown that by carefully manipulating the drug-to-polymer ratio and the molecular weight and composition of the polymer, the extent and level of drug release from the nanoparticle can be finely tuned in addition to the amount of nanoparticle that is taken up into cells.[68-70] Examples of synthetic biodegradable polymeric nanoparticles include poly(ε-caprolactone), polylactic acid (PLA), poly(lactide-coglycolide) (PLGA), polyglycolic acid, and poly(alkylcyanoacrylate). Natural polymers include gelatin, dextran ester, and chitosan; however, they do not have as high purity and reproducibility as the synthetic polymers. Polymeric nanoparticles have therefore been intensively investigated as drug delivery systems over the last decade, with the FDA approving biodegradable polymeric nanoparticles such as PLA and PLGA for human use.[63] The use of paclitaxel bound to a natural polymeric nanoparticle made from albumin has been also been approved by the FDA for the treatment of patients with metastatic breast cancer,[23] whereas a polymeric nanoparticle formulation of docetaxel is currently under phase 1 clinical trials in patients with advanced solid malignancies (clinicaltrials.gov identifier NCT00103791).

Mesoporous silica nanoparticles have also been extensively studied for their ability to provide a physical encasement to protect and house a drug from degeneration or denaturation. The surface pores on the mesoporous nanoparticle could either lead to a central reservoir that contains a drug payload or the nanoparticle itself could be formed of a complex worm-like network of channels allowing for the delivery of relatively large doses of a drug in a controlled manner.[71] It has also been shown that the distribution of pore sizes determines the release kinetics of the drug payload.[72] There have also been studies examining reversibly capping the surface pores of mesoporous silica nanoparticles to physically block the release of drugs until the nanoparticle has reached its target with "zero premature release." Examples of nanoparticle caps include cadmium sulfide, whose disulfide links are chemically cleavable by disulfide-reducing agents,[73] and iron oxide nanoparticle caps, which are released from mesoporous silica nanorods in the presence of an external magnetic field.[74] Such carrier systems are also able to deliver membrane-impermeable drugs, thereby serving as a universal transmembrane carrier for intracellular drug delivery and imaging applications.[73,75,76]

Inorganic nanoparticles encompass a vast range of nanoparticle platforms synthesized from metals, metal

oxides, and metal sulfides. They can be produced with a plethora of different designs varying in size, shape, and porosity with great reproducibility and can be easily conjugated with ligands for tumor targeting and/or with chemotherapeutics for tumor therapy. In addition, their surface composition can also be easily manipulated to create nanoparticles that can evade the RES. Furthermore, when compared with liposomes and solid lipid nanoparticles, they are relatively stable over large ranges of pH and temperatures. However, their lack of biodegradation and slow dissolution rates raise concern and uncertainty regarding their degradation and elimination from the body.

The Stability of the Nanoparticle-Drug Complex

Blood circulation times are determined by the clearance rate through renal excretion and interactions with the RES. Nanoparticles that are relatively small are rapidly cleared by the kidneys, whereas larger nanoparticles are cleared by the RES.[63,77] Entrapment of nanoparticles by cells of the RES reduces their systemic bioavailability. However, surface modification with hydrophilic PEG chains can give nanoparticles "stealth-like" characteristics, resulting in their prolonged presence in the circulation by reducing their immunogenicity and inhibiting their recognition and phagocytosis by mononuclear phagocytic cells.[78] In addition, as "naked" nanoparticles adsorb proteins, which makes them aggregate in biological media, PEGylation of nanoparticles serves as a biological layer to improve stability and reduce potential protein adsorption and aggregation. This prevents nanoparticle aggregation in solution, which helps keep them from forming a cluster once in blood vessels, where they could otherwise embolize and occlude blood flow resulting in microinfarctions at distant sites and organs.[63]

Delivery of the Nanoparticle-Drug Complex to Tumor Cells

After administration into the systemic circulation, nanoparticles can be delivered to tumors either passively or actively. In passive delivery, nanoparticles are able to exploit the unique EPR effect of tumors, which enables them to leave the systemic circulation and enter the extravascular space, where they can accumulate around tumor cells.[10] To take advantage of this effect, nanoparticles should ideally be less than 100 nm in size.[79] However, the localization of nanoparticles within the tumor will not be homogenous due to heterogeneity in tumor blood supply and interstitial flow and physiological barriers such as the density of the interstitial matrix.[80] Alternatively, nanoparticles can be actively targeted to tumors with the help of surface modifications (ie, the addition of ligands such a peptides, small molecules, oligosaccharides, antibodies, and affibodies)[81,82] that allow the nanoparticle to recognize and bind to complementary target molecules on the surface of tumor cells. The target molecule can be a receptor or antigen, but must be expressed at high levels on the surface of tumor cells and at low or negligible levels on normal cells. By specifically targeting tumor cells, nanoparticles are able to increase the delivery of the drug to target cells while concurrently reducing the toxicity of the free drug to nontarget organs, thereby increasing the therapeutic index of the drug.[83] Studies have also shown that binding to multiple receptors simultaneously can result in nanoparticles displaying multivalent characteristics and hence stronger interactions with the surface of malignant cells. Examples include nanoparticles with bound folate ligands that demonstrated a 10-fold higher affinity for the folate-binding protein than free folate due to folate receptors often being found in clusters on the surface of malignant cells.[84] In addition, the PEG chains added to nanoparticles to improve their biocompatibility can also be functionalized by serving as linking conduits for tumor-specific ligands.

Release of the Drug From the Nanoparticle-Drug Complex

Once delivered to the site of the tumor, the nanoparticle-drug complex must dissociate to release the drug. Upon binding to cancer cells, drugs are released from nanoparticles either by diffusion out of the matrix or by swelling, erosion, or degradation of the nanoparticle.[85] For example, a new nanoparticle carrier has recently been developed that undergoes reversible volume change upon phototriggering with ultraviolet light to allow the release of chemotherapeutic drugs, thereby providing spatiotemporal control of drug release.[86] However, the limited tissue penetration of ultraviolet light may somewhat impede the clinical translation of this new technology. Other systems include hybrid nanoparticle designs that allow a multistage delivery system. Here, the different layers of the nanoparticle each respond differently to the surrounding biological environment such that changes in oxidative stress, temperature, or pH (ie, in the acidic microenvironment of a tumor)[87] will result in changes in the nanoparticle configuration, thereby allowing the release of preloaded chemotherapeutic drugs.[88] Another nanoparticle system currently under development includes a 100-nm nanoparticle that shrinks to 10 nm once it reaches the tumor for more efficient diffusion through the interstitial space, thereby allowing enhanced permeation and deeper penetration into the tumor tissue.[89] This size change is triggered by proteases that are highly expressed in the tumor microenvironment, such as matrix metalloproteinases-2, which degrade the cores of the 100-nm gelatin nanoparticle.

Such characteristics are valuable in treating tumors that accumulate fibrillar collagen types I and III in the interstitial spaces, which stiffen the extracellular matrix and induce fibrosis, thereby hindering the diffusion and penetration of larger nanoparticles.[90,91] The ability of nanoparticles to allow controlled and sustained drug release from their matrix also overcomes issues related to the release of drugs at predetermined rates, irrespective of the patient's needs or the constantly changing tumor environment. Using a system in which drug release can be triggered enables drug concentrations to be maintained within their therapeutic range for longer periods of time, as well as allowing repeated dosing from a single administration. It has also been suggested to be valuable in allowing "chrono-administration," in which the specific timing of chemotherapeutic drug delivery has been hypothesized to be critical in achieving an optimal therapeutic effect to maximize tumor killing and minimize metastatic spread.[86,92]

After release from their nanoparticle carrier, the next challenge for most chemotherapeutic drugs is to be delivered inside cancer cells for them to have a therapeutic effect on intracellular targets. Therefore, there is a competition between how fast the drug can enter the cell, through either active transport mechanisms or via receptor-mediated endocytosis, and how quickly it will diffuse away from the cancer cell. Hence, to increase the efficiency of drug delivery to appropriate intracellular targets, several groups are also developing strategies whereby the nanoparticle-drug complex can enter inside cancer cells before releasing pharmaceutical agents into the cytosol. Labeling nanoparticles with cell-penetrating peptides, including Penetratin, a cell-penetrating peptide[93]; transactivator of transcription (TAT) peptide[94]; anti–actin-targeting molecules[95]; and sweet arrow peptide[96] will encourage their intracellular uptake. This is likely to have a greater therapeutic effect as most agents have primary targets within cells.[63] Furthermore, this is especially beneficial for those drugs that are rapidly exported from cells via efflux transporters such as multidrug resistance-associated protein pumps,[63,97] since it has been shown that P-glycoprotein most likely works by recognizing drugs that are to be effluxed out of cells only when they are present in the plasma membrane but not when they are located in the cytoplasm or lysosomes after endocytosis.[2,98] Once inside the cell, the chemistry of the nanoparticle can assist in releasing the drug. For example, if drugs are conjugated to nanoparticles with thiol groups, these can be exchanged with glutathione (which is abundant in the cytoplasm), resulting in the release of any bound drugs.[99,100] In cases in which the nanoparticle-drug complex is not internalized as an entire entity into the cancer cell, the drug could instead be released from the nanoparticle outside the cell, where it may then enter the cell through simple diffusion or other transport systems. The disadvantage of this mechanism of drug delivery is that some of the drug may be redistributed to surrounding normal tissues, thereby decreasing its therapeutic effectiveness.[10] In addition, as the surrounding interstitial environment of the tumor is acidic, it also creates a hostile microenvironment for drug delivery as well as inhibiting the efficacy of alkaline chemotherapeutic drugs.

Removal of the Residual Nanoparticle After Drug Release

Most nanoparticle-drug systems that have been developed have been made of biodegradable materials (ie, phospholipids, lipids, dextran, and chitosan), which allow the release of the drug after degradation of the nanoparticle carrier. However, nonbiological carriers, such as inorganic nanoparticles, are relatively stable over ranges of temperature and pH and this raises concerns regarding their lack of biodegradation after the drug delivery. Hence, if these nonbiological materials are used, they must have a way of being safely removed from the body or processed and stored in a stable state within the body (ie, within inactive macrophages). By carefully controlling the chemistry of the nanoparticles during their synthesis, nanoplatforms can also be designed whereby the nanoparticle can disassociate into its basic structural components, which are not likely harmful after drug delivery.

Nanoparticles as Therapeutic Agents

Photodynamic Therapy

Photodynamic therapy (PDT) has recently emerged as a viable therapeutic option in the treatment of cancer. PDT uses a light-activatable chemical known as a photosensitizer, which absorbs light of a certain wavelength to generate cytotoxic oxygen-based molecular species. These reactive species cause damage to subcellular organelles and plasma membranes, resulting in cell death either by apoptosis, necrosis, or autophagy. Photosensitizers are able to transfer the energy they have absorbed from light to either oxygen molecules to produce singlet oxygen or to surrounding molecules to form free radicals, which can subsequently react with molecular oxygen to produce superoxide, hydrogen peroxide, and hydroxyl radicals. The effectiveness of PDT depends largely on the efficiency with which photosensitizers can generate singlet oxygen production and their ability to be selectively delivered at therapeutic concentrations to the target tumor tissue.[101] As singlet oxygen species have a short lifespan of less than 3.5 microseconds and can diffuse only 0.01 to 0.02 μm, their extent of damage is limited to the site where the photosensitizer molecules accumulate, which usually is in the mitochondria or endoplasmic reticulum.[102,103] Because many

photosensitizers absorb light in the visible spectral region below 700 nm, the depth penetration of light is limited to only a few millimeters, thereby only allowing the treatment of relatively superficial lesions. However, advances in optical engineering have enabled the development of optical fibers that can be incorporated into endoscopes, bronchoscopes, and colonoscopes to allow for the delivery of light to internal body cavities, thereby extending the scope of PDT. Currently, PDT is being explored in the treatment of several cancers including skin,[104] bladder,[105] prostate,[106] lung,[107] esophageal,[108] pancreatic,[109] stomach,[110] and head and neck[111] cancer to name a few.

Nanoparticles used in PDT can functionally be classified as either passive or active (Fig. 3). Passive PDT nanoparticles are carriers for photosensitizers and can be made from either biodegradable material or non–polymer-based materials such as ceramic and metallic nanoparticles. Biodegradable nanoparticle carriers, made from PLGA or PLA, have been shown to provide an alternative solution to liposomes due to their ability to encapsulate photosensitizers with high carrier capacity. This is important as photosensitizers are highly hydrophobic with inherent poor water solubility, resulting in aggregation in solution that limits their ability to be parentally administered. In addition, the morphology and composition of the polymer matrix can be optimized for the controlled degradation of the polymer and hence release of the photosensitizer molecules. Photosensitizer-loaded nanoparticles have been shown to have higher photoactivity than "free" photosensitizers. Furthermore, smaller nanoparticle carriers have a greater phototoxic effect compared with larger carriers due to their higher rate of intracellular uptake via endocytosis, resulting in the release of photosensitizers within the cytosol and not the extracellular environment. In addition, the smaller the nanoparticle size, the larger the surface area-to-volume ratio, which increases the surface area exposed to the surrounding medium, thus resulting in higher photosensitizer release rates.[112] Nonbiodegradable materials can also be loaded with photosensitizers and have advantages over organic polymeric nanoparticles, including stability; exquisite control over size, shape, and porosity; and immunity to changes in pH and microbial attack. In addition, they can be easily functionalized for selective targeting of tumor tissue, which will allow for the selective accumulation of photosensitizers at the site of cancer while reducing the accumulation of photosensitizers in nontarget normal tissues. This will therefore lower the concentration of photosensitizers used to generate the same phototoxic effect, thereby increasing the phototherapeutic index. Two photon absorption dyes can convert low-energy radiation into higher-energy emissions, which can be directly transferred to molecular oxygen to generate singlet oxygen. The advantage of this system is that it can be activated in deep

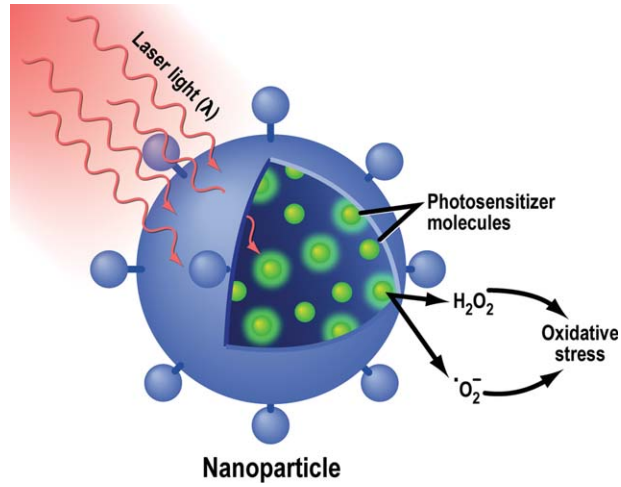

FIGURE 3. Nanoparticles in Photodynamic Therapy. Nanoparticles can deliver light-activatable chemicals, known as photosensitizer molecules, to tumor cells for use in photodynamic therapy. After the absorption of light, photosensitizer molecules can generate cytotoxic oxygen-based reactive species, which can subsequently cause cellular damage and cell death via oxidative stress.

tissues by light in the tissue transparent window (750-1000 nm), which has deeper tissue depth penetration. Nevertheless, the dye's toxicity remains a major problem. Entrapping the dye in a nanoparticle carrier, which is biologically inert, can therefore reduce its toxicity to normal tissue while allowing PDT penetration in deeper tissues. Other groups are also exploring the use of exciting photosensitizers (energy acceptors) indirectly through fluorescence resonance energy transfer from photon absorbing dyes (energy donors).[113] By physically encapsulating the dye and the photosensitizer in the same nanoparticle, this approach allows for the efficient transfer of energy between the dye, which acts as an intermediary, and the active coencapsulated photosensitizer. For efficient photon excitation using this concept, the loading density of the energy-donating photon absorption dye needs to be much higher than that of the energy-accepting photosensitizer. Hence, modified silica nanoparticles have been used as they are biocompatible, stable without releasing encapsulated hydrophobic molecules, and suitable for PDT because their porous matrix is permeable to oxygen molecules.[114]

Active PDT nanoparticles can themselves generate reactive species without the presence of a photosensitizer. This was first appreciated by Samia et al, who found that in addition to sensitizing photosensitizer molecules through a fluorescence resonance energy transfer, semiconductor QDs could themselves generate singlet oxygen alone via a triplet energy transfer without the need for photosensitizers, albeit with a lower efficiency.[115] Other groups have also investigated the ability of nanoparticles to play an additional active intermediary role in the process of PDT, in addition to encapsulating photosensitizers and targeting them to cancer cells.[101] These nanoparticles will emit luminance of an appropriate wavelength to active

photosensitizers after irradiation with x-rays, thereby offering therapy to regions deep within the body that can be reached with ionizing radiation. Similarly, upconverting nanoparticles are able to take low-energy radiation (ie, near-infrared radiation [NIR], which can penetrate tissue depth of approximately an order of magnitude more than visible light) and generate higher-energy light that can activate photosensitizers to produce singlet oxygen from dissolved molecular oxygen in the microenvironment. This is done following the simultaneous absorption of 2 low-energy photons, allowing the nanoparticle to transition from a ground to an excited state through the use of a transition metal or a rare earth ion such as lanthanide. Quantum mechanically, this takes place via a virtual intermediate state following the absorption of the first photon.[101]

Gene Silencing

Gene therapy involves using plasmid DNA, antisense oligonucleotides, or small interfering RNA (siRNA), the latter of which requires the lowest dose for gene regulation.[116] siRNA is formed from the cleavage of double-stranded RNA by "dicer," which is an ribonuclease (RNase) III endonuclease. siRNAs are short double-stranded RNA fragments measuring approximately 20 to 25 nucleotides in length and have the ability to interfere with the translation of specific mRNAs complimentary to its nucleotide sequence.[117] siRNAs interact with a multifunctional protein called Argonaute, which is the catalytic component of the RNA-induced silencing complex. Here, duplex siRNA is unwound and Argonaute degrades the passenger RNA strand, thereby allowing the remaining template/antisense strand to bind a complementary mRNA. Argonaute then cleaves the mRNA through its endonuclease activity, leading to silencing of gene expression, otherwise known as RNA interference (RNAi).[118] This effect may last for 3 to 7 days in rapidly dividing cells or for many weeks in nondividing cells.

As the mechanisms underlying cancer become better defined, multiple molecular targets are being identified. siRNA therefore holds great promise in being able to silence not only one but several genes that contribute to cancer progression with high efficacy and specificity, thereby allowing the simultaneous targeting of multiple pathways. Several in vitro and in vivo studies have investigated RNAi in pathways that drive cancer, such as apoptosis, cell cycle regulation, cell senescence, and tumor-host interactions, with promising results.[119,120] However, there are several limitations that have been highlighted by Miele et al that reduce the therapeutic efficacy of siRNA, including 1) delivery problems, 2) side effects due to off-target actions (ie, partial pairing of siRNA with the complimentary sequence from unintended nontarget mRNA transcripts), 3) disturbance of physiological functions of the cellular machinery involved in gene silencing, and 4) the induction of the innate immune response mediated by type 1 interferon and proinflammatory cytokines.[85]

Probably the most significant of these problems is the ability to deliver a sufficient amount of siRNA into the cytoplasm of target cells after systemic delivery. Unmodified siRNA molecules are highly unstable when delivered into the systemic circulation, with a short half-life due to serum RNase A-type nucleases and rapid renal clearance. Furthermore, unmodified siRNA molecules are unable to enter cells due to their size and highly polyanionic charge of the phosphate backbone, which results in electrostatic repulsion from the anionic charge of the cell membrane surface. Although chemical modifications of siRNA have been shown to improve intravascular stabilization and reduce activation of the innate immune response without significant loss of RNAi activity, other delivery systems such as nanoparticles are currently being explored as an alternative way to safely transport siRNA. Nanoparticles have a large surface area-to-volume ratio, thereby providing an enormous surface area for the transport of siRNA relative to their small volume (Fig. 4). Nanoparticles can carry and protect siRNA following intravenous administration in addition to specifically targeting and delivering siRNA to cancer cells after functionalization with tissue-specific ligands. Nanoparticles are efficiently taken up into cells, usually via the endosomal pathway through membrane fusion or receptor-mediated endocytosis. Once inside target cells, they enter the intracellular trafficking pathway, at which point the siRNA must escape before the lysosome degrades the RNA. Fusogenic lipids and proteins, photosensitive molecules, and pH-sensitive lipoplexes and polyplexes are some of the mechanisms used to improve endosomal escape.[121]

Of all the siRNA-nanoparticle delivery systems being developed, nanoliposomes are probably the closest to being clinically translated. Nanoliposomes are made from biological material and consist of a phospholipid bilayer and an aqueous core that can hold and interact with siRNA through complexes that are stabilized by electrostatic interactions.[122] They are typically neutral in charge and approximately 30 to 40 nm in size, thereby enabling their efficient uptake into cells. Nanoliposomes protect siRNA in the circulation from endonuclease activity; however, their short half-life in serum and rapid clearance from the circulation by the RES (ie, the liver, spleen, lung, and bone marrow) limit their use as treatment and will require a continuous infusion or frequent administration. Several groups are currently investigating the potential use of sustained-release polymer formulations to overcome this problem.[85,123] Solid lipid nanoparticles are also being

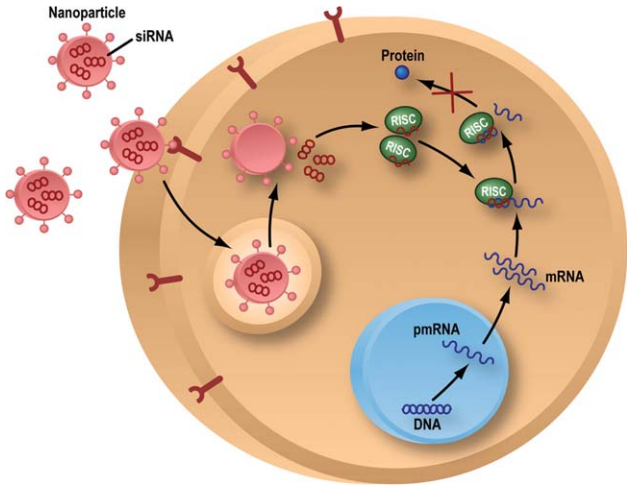

FIGURE 4. Precursor mRNA (pmRNA). Nanoparticles in RNA Interference Gene Therapy. Nanoparticles can deliver small interfering RNAs (siRNAs) into tumor cells, where they can interfere with the translation of specific messenger RNA (mRNA) molecules. siRNA interacts with a multifunctional protein called Argonaute, which is the catalytic component of the RNA-induced silencing complex (RISC). Here, duplex siRNA is unwound and Argonaute degrades the passenger RNA strand, thereby allowing the remaining template/antisense strand to bind to a complementary mRNA. Argonaute then cleaves the mRNA through its endonuclease activity, leading to silencing of gene expression, otherwise known as RNA interference. pmRNA indicates precursor mRNA.

investigated as they are prepared from physiological lipids and therefore have excellent biocompatibility and minimal biological toxicity.

Nonbiological or synthetic nanoparticles, such as inorganic crystals and noble metals, have also been explored as gene delivery vehicles due to their increased stability and ability to be easily functionalized with oligonucleotides. The optimum size of synthetic nanoparticle carriers appears to lie between 5 and 100 nm because nanoparticles measuring less than 5 nm undergo rapid renal clearance while those measuring greater than 100 nm are taken up by the RES, where they are degraded by activated monocytes and macrophages. Furthermore, particles measuring greater than 200 nm activate the complement system and are therefore cleared more efficiently and rapidly than smaller nanoparticles.[124] Incorporation of siRNA into gold nanoparticles was first accomplished by Oishi et al with newer advances using a layer-by-layer assembly to create supramolecular structures to allow for the sustained release of siRNA.[125] However, as chemical modification of both the carrier surface and the transported drug is required, new delivery strategies based on porous silicon are being developed.

In 2010, Davis et al undertook the first proof-of-principle study in which siRNA designed to reduce expression of the M2 subunit of ribonucleotide reductase (RRM_2) was packaged into a nanoparticle containing a linear cyclodextrin-based polymer, a human transferrin protein to engage transferrin receptors on the surface of the cancer cells, and PEG to promote nanoparticle stability. In a small phase 1 clinical trial, these nanoparticles packed with siRNA targeted against RRM_2 were administered systemically on days 1, 3, 8, and 10 every 21 days via a 30-minute intravenous infusion to patients with melanoma that was refractory to the standard of care. Tumor biopsies in a limited number of patients following treatment showed nanoparticles within the intracellular compartment with corresponding reductions in both RRM_2 mRNA and protein levels, demonstrating that siRNA systemically administered to humans can produce a specific gene inhibition via an RNAi mechanism.[51] However, little is still known about the pharmacodynamics of the RNAi effects, which rely on a combination of nanoparticle disassembly time and the time that the siRNA resides within the RNAi machinery. Several other early clinical trials are currently underway investigating the use of siRNA in chronic myeloid leukemia, liver tumors, neuroblastoma, and advanced solid malignancies.[85]

Photothermal Therapy

Hyperthermia refers to temperatures between 40°C and 45°C. Temperatures greater than 42°C have been shown to make cancer cells more susceptible to the effects of additional treatments such as irradiation, in addition to causing a degree of apoptosis, while temperatures above 45°C can cause direct cell death (ie, thermoablation).[126,127] Hyperthermic treatment of tumors involves heating tumors using radiofrequency (RF), microwaves, magnetic fields, or ultrasound to cause irreversible cellular damage by loosening membranes and denaturing proteins, which ultimately results in cell death. Although this effect is more selective for tumors due to their reduced heat tolerance, thermal therapy has been limited by damage caused to surrounding normal tissue.[128] Photothermal therapy (PTT) aims to overcome this problem by using photothermal agents to achieve more controlled and selective heating of the target area, thereby confining thermal damage to the tumor.

For photothermal agents to be effective, they need to have an enhanced light absorption and efficient light-to-heat conversions.[129] Traditional agents include natural chromophores, which suffer from low absorption, or external dyes (ie, indocyanine green), which suffer from photobleaching. However, the development of noble metal nanoparticles (ie, gold nanospheres, nanorods, nanoshells, and nanocages) and carbon nanotubes has overcome these problems as they have strong absorption in the NIR regions of the electromagnetic spectrum, especially at 650 to 900 nm, due to surface plasmon resonance (SPR).[130] This is advantageous as most biological tissues exhibit minimal light absorption in this range, thereby allowing for increased depth penetration of light. Generally, spherical gold nanoparticles have their maximal SPR absorption

peak in the visible spectrum, around 520 nm, without much tunablity of this peak. In contrast, gold nanorods have 2 absorption bands along each direction of the rod (ie, the longitudinal and transverse axes), with the transverse plasmon band showing a strong absorption peak at approximately 520 nm and the longitudinal plasmon band located at higher frequency, which can be tuned in the NIR region depending on their length-to-width ratio, thereby making them attractive for in vivo PTT. Similarly, the SPR absorption peak for gold nanoshells can be tuned by altering their shell thickness-to-core radius ratio.[130,131] Due to the SPR of nanoparticles, their absorption coefficients are 4 to 5 orders of magnitude higher than those offered by photothermal dyes.[130,132] Photoexcitation of metal nanoparticles with light frequencies that overlap with the nanoparticle SPR absorption band results in the formation of a heated electron gas that subsequently cools rapidly within approximately 1 picosecond (ps) by exchanging energy with the nanoparticle lattice. The lattice then cools by exchanging heat with the surrounding environment within approximately 100 ps to cause localized tissue destruction.[133] In addition to the mechanisms of heat-induced cellular destruction described above, the heating of gold nanoparticles also causes cavitation bubble formation around the nanoparticle, which in turn results in mechanical stress leading to cell damage.[134]

Studies have shown that nanoparticles generally have a better light-to-heat conversion compared with conventional dyes, thereby requiring lower laser energies to achieve local cellular destruction.[128,135] To increase the efficiency of the light-to-heat conversion, nanoparticles are required to be in the size range of tens to hundreds of nm; however, this results in their poor clearance and accumulation within the RES. Hence, studies are currently looking at using smaller noble metal nanoparticles that can evade the RES but that aggregate at the site of the tumor through self-assembly. The loading of nanoparticles on tumor cells will increase the optical density thereby resulting in lower laser powers required to raise the temperature above the threshold needed for cellular destruction.[135]

For PTT to be effective, photosensitizer nanoparticles need to initially accumulate within the target tumor following intravenous or local administration. This can be achieved by functionalizing the nanoparticles with specific tumor-targeting molecules (Fig. 5). For example, cell culture studies have shown that anti-epidermal growth factor receptor (anti-EGFR) antibody–conjugated gold nanoparticles specifically bind and load onto cancer cells expressing EGFR to enable PTT by allowing suitable wavelength laser pulses to generate temperatures of approximately 70°C to 80°C, leading to necrotic cell death from thermal ablation. By contrast, no photothermal destruction was observed for cell types that had no nanoparticle labeling, even at 4 times the energy required to kill the malignant cells labeled with anti-EGFR–gold nanoparticles.[135] The next step is to deliver light specifically to the tumor region, which is usually undertaken by using NIR laser probes within endoscopes or fiber optic catheters that can be positioned adjacent to the tumor. The exciting results of PTT in cell culture, ex vivo human specimens, and living animal models demonstrates great promise for this cancer therapy strategy, either alone or in combination with other treatment modalities. Early clinical trials are currently underway using NIR PTT for refractory head and neck cancers with AuroShell nanoparticles, which consist of a gold metal shell and a nonconducting, or dielectric, silica core (NCT00848042).

Iron oxide nanoparticles in water have also been shown to generate heat when injected directly into tumors in the presence of an externally applied oscillating magnetic field.[136] As iron nanoparticles within water (ie, magnetic fluids) have a high particle density per volume resulting in a large overall surface area of magnetic elements, this results in excellent power absorption capabilities making them eminently suitable for contactless, selective interstitial heating of tumors.[137] In models of prostate cancer,[138] malignant glioma,[139] and breast cancer,[140] magnetic fluid hyperthermia has shown promising results with phase 1 clinical trials for prostate cancer and phase 2 clinical trials for brain cancer that are currently underway.[126] At present, magnetic fluid hyperthermia cannot be achieved with systemic injection of iron oxide nanoparticles.

Nanoparticles as Imaging Agents

Conventional imaging using plain radiographs, ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI) has traditionally been used in both cancer screening and follow-up. However, all these modalities rely on detecting cancer once it becomes a visible physical entity, at around 1 cm^3 at which point the tumor mass will already contain approximately 1 billion cancer cells.[141] Over the past decade, there has therefore been a paradigm shift from anatomical imaging, which detects macroscopic/gross pathology, to molecular imaging, which has the potential to detect cancer much earlier at the molecular level, long before phenotypic changes occur. Molecular imaging allows the genetic changes involved in oncogenesis to be characterized in vivo, thereby predicting the type of molecular therapy that will prove most beneficial for the patient (ie, personalized medicine). It also allows the repeated noninvasive monitoring of the disease for response, progression, and transformation following therapy or recurrence.

While traditional imaging modalities have the option of using imaging agents to highlight existing features (ie, blood vessels and tissue perfusion following intravenous

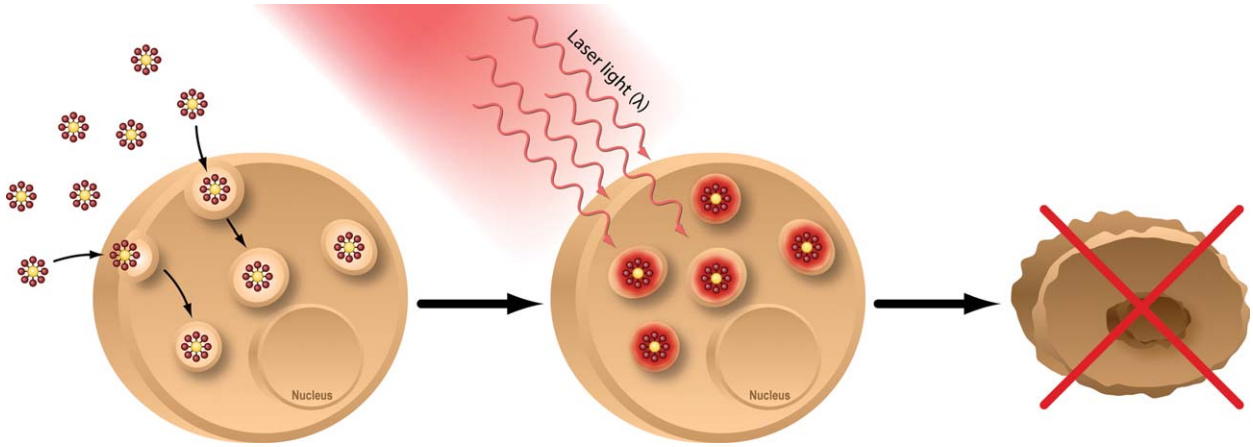

FIGURE 5. Nanoparticles in Photothermal Therapy. Nanoparticles can be used in photothermal therapy to cause localized destruction of tumors after absorption of light due to their efficient light-to-heat conversion. The controlled and selective heating of nanoparticles allows thermal damage to be confined to the tumor while minimizing any damage to surrounding normal tissue.

contrast medium), molecular imaging techniques must use imaging agents. Traditionally, small molecules, which measure approximately less than 2000 daltons and approximately 1 nm, have routinely been used as imaging agents in clinical practice (ie, 2-deoxy-2-(^{18}F)fluoro-D-glucose [FDG] for positron emission tomography [PET], iodinated small molecules for CT, and chelated gadolinium for MRI). However, their low signal intensity, poor stability, nonspecific interactions, and rapid clearance from the circulation have led to the development of newer probes. Nanoparticles have shown great promise in overcoming these limitations and are currently being developed as molecular imaging agents (Table 2).[57,142-144] For example, when using optical imaging modalities, nanoparticles can increase signal intensity, thereby allowing fewer numbers of cells to be imaged at greater tissue depths, as well as providing imaging signals that are stable over longer periods of time.

Nanoparticles also have a high avidity as they can be coated with multiple copies of ligands, which will allow multiple bond interactions with cellular target moieties, thereby increasing their association constant by 4 to 5 orders of magnitude.[145] This is advantageous as it will allow more nanoparticles to accumulate at the site of the tumor, thereby increasing the signal-to-noise ratio, which allows cancerous tissue to be better highlighted relative to adjacent normal tissue. Most nanoparticle imaging agents are also larger than 10 nm and are therefore not typically cleared renally from the circulation[146]; this allows them to have longer circulation times when compared with small molecules (ie, days vs minutes). which is useful as it allows repeated imaging without the need for further nanoparticle administration. Interestingly, studies have also shown that smaller nanoparticles have a more uniform tissue biodistribution and that nonspherical nanoparticles (ie, nanodisks, nanotubes, nanoworms, etc) are more efficiently delivered to target areas when compared with spherical nanoparticles.[147,148] However, this has to be balanced against the potentially increased toxicity that is associated with nonspherical nanoparticles.[149,150] As cancer is rarely caused by a single molecular alteration, simultaneously detecting multiple molecular targets that are upregulated during oncogenesis (ie, a phenomenon known as multiplexing) will increase the specificity of cancer detection. One way to do this is to label different nanoparticles, each against a single molecular biomarker target, and then administer all these nanoparticles at once. The signals detected from the different nanoparticles bound to the cancer cells can then be decoded to allow a molecular profile of the cancer to be determined. In turn, this will enable a molecularly targeted therapy to be designed and administered to the patient. An alternative strategy if the molecular profile of the cancer is already known is to label a single nanoparticle with multiple different ligands, each directed at a different molecular target know to be upregulated by the tumor being investigated. As the tumor will contain more of these targets compared with background tissue, it will bind more nanoparticles thereby generating a stronger signal. Finally, nanoparticles are able to be designed to be multimodal such that they can be imaged by 2 or more different imaging modalities (eg, fluorescence and MRI). To increase the delivery efficiency of nanoparticle imaging agents to the tumor bed, several groups are also currently researching ways of individually injecting the subcomponents or building blocks of a nanoparticle. In the presence of certain triggers such as pH adjustment, reduction, or enzyme cleavage, these subcomponents are then able to self-assemble to create a supramolecular nanoparticle probe that can then be used for imaging.[151,152] The advantage of this approach is that the individual subcomponents will be smaller and hence have better access to the tumor thereby maximizing accumulation

TABLE 2. Examples of Nanoparticles Used in Cancer Imaging

IMAGING MODALITY	DESCRIPTION OF NANOPARTICLE	CANCER IMAGED BY THE NANOPARTICLE	STAGE OF DEVELOPMENT/CLINICAL TRIAL NO.
MRI	Superparamagnetic iron oxide nanoparticles	Liver tumors (ie, hepatocellular carcinoma, liver metastases)	Currently used in clinical practice[142]
		High-grade glioma	NCT00769093
	Ultrasmall superparamagnetic iron oxide nanoparticle	Preoperative staging of pancreatic cancer	NCT00920023
		Pelvic lymph node metastases from prostate, bladder, or other GU cancers	NCT00147238
CT	Heavy metal (ie, gold, lanthanide, and tantalum) nanoparticles	Solid organ tumors	Preclinical stage of development[143]
SPECT	TC-99m sulfur colloid nanoparticles	Sentinel lymph node mapping in invasive breast cancer	NCT00438477
PET	^{124}I-labeled cRGDY silica nanoparticles	Melanoma and malignant brain tumors	NCT01266096
Optical	Surface-enhanced Raman scattering nanoparticles	Colorectal cancer	Preclinical stage of development[57]
Photoacoustic	Single-walled carbon nanotubes	Solid organ tumors	Preclinical stage of development[144]

MRI indicates magnetic resonance imaging; NCT, National Clinical Trial; GU, genitourinary; CT, computed tomography; SPECT, single-photon emission computed tomography; TC-99m, technetium-99m; PET, positron emission tomography; ^{124}I, iodine-124; cRGDY, cyclic Arg-Gly-Asp-Tyr.

at the target site. Examples include gadolinium-containing monomers that assemble in cells via thiol-sensitive reduction of 1,2-aminothiol and 2-cyanobenzothiazole and probes with a motif sensitive to proteases such as furin and caspase-3, which are overexpressed in tumor cells.[153]

Although a lot of work is currently being undertaken preclinically to develop new nanoparticle agents, superparamagnetic iron oxide nanoparticles (SPIONs) are already being used in clinical practice for hepatic, cardiovascular, cellular, and lymphatic imaging. Iron oxide (magnetite, Fe_3O_4; maghemite, Fe_2O_3) nanoparticles become superparamagnetic at room temperature if their core diameter is 20 nm or less,[154] which allows for susceptibility effects at micromolar concentrations that modify the T2 and T2* relaxation times of water protons for enhanced MRI contrast.[155] SPIONs are also considered to have low toxicity in vivo as they are thought to be biodegradable, with the iron from the nanoparticles released upon degradation into the normal plasma iron pool, where it can subsequently be incorporated into hemoglobin in erythrocytes or used for other metabolic processes.[156,157] SPIONs have been used to characterize liver lesions since they are phagocytosed by cells of the RES. As normal liver parenchyma contains RES, they will accumulate SPIONs, resulting in a decrease in signal intensity on both T2-weighted and T1-weighted images. In contrast, most liver tumors do not contain RES and hence they will not uptake SPIONs, thereby improving contrast between the tumor (high signal) and the surrounding tissue (low signal).[158] However, these signal characteristics are reversed when SPIONs are combined with ligands for active targeting.[159] In these circumstances, SPIONs will now accumulate at the site of the tumor, resulting in a low signal compared with the background liver parenchyma; however, this relies on SPIONs avoiding the RES. To avoid the RES and improve colloidal stability and biocompatibility, SPIONs used for active targeting are usually coated with a polymer (ie, dextran, starch, or PEG).[159] Ligands such as folate are then conjugated to SPIONs via their polymer coatings of either dextran[160,161] or PEG.[162] Folate has been used as a ligand since folate receptors are expressed in limited quantities on the apical surfaces of normal epithelial cells but are generally overexpressed in cancerous tissues due to the vital role that folate plays in cellular proliferation. Transferrin has also been covalently coupled to SPIONs[163] as it will bind to the transferrin receptor (also known as CD71), which is a type II transmembrane glycoprotein that is overexpressed on the surfaces of proliferating cancer cells because of their increased iron requirements.[164] SPIONs have also been combined with peptide sequences such as arginyl-glycyl-aspartic acid (RGD),[165] which can combine with integrins such as $\alpha_v\beta_3$ that are expressed on the surface of proliferating endothelial cells such as those undergoing angiogenesis.[166] Initially, SPIONs conjugated with monoclonal antibodies were not considered practical for in vivo diagnostics due to the large particle size, which facilitated their rapid clearance by the RES.[159] However, this has proved not to be the case, with several studies showing monoclonal antibody-conjugated SPIONs having strong specificity for antigen-expressing tissues. Antibodies against EGFR have been conjugated with SPIONs for the detection of colorectal, small cell lung, and esophageal squamous cell carcinomas in experimental models.[167-169]

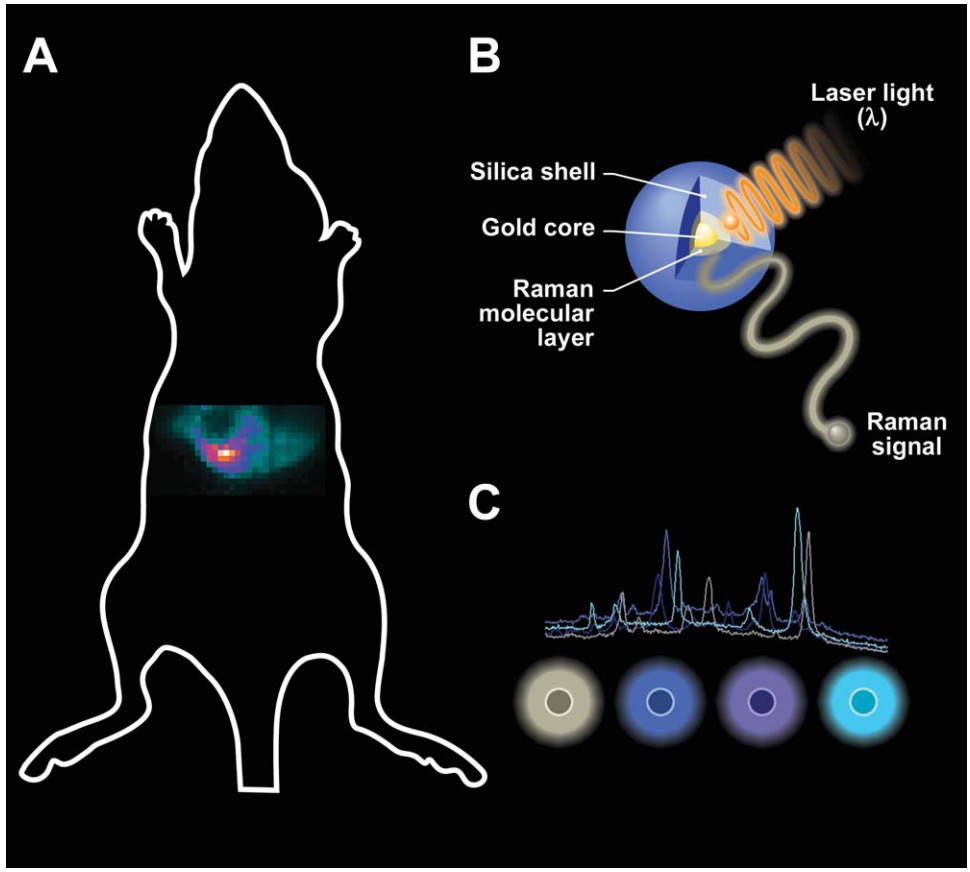

FIGURE 6. Raman Nanoparticles Used in Image Multiplexing. (A) An example of a spectral intensity map from Raman nanoparticles targeting a tumor in a xenograft mouse model. (B) A schematic representation of a Raman nanoparticle. After activation of the Raman molecular layer with a laser light of a specific wavelength, the Raman nanoparticle emits a Raman signal/spectral trace that can be subsequently detected. (C) An example of 4 individual Raman spectral traces as a result of different Raman molecular layers in 4 different Raman nanoparticles.

Nevertheless, the greatly increased size of the nanoparticle-antibody complex does result in reduced stealth-like characteristics. Hence, some groups are now conjugating SPIONs with aptamers, which are artificial, very small, selected oligonucleotide sequences that can bind ligands with very high specificity and affinity.[170] There are also dual-modality probes being developed such as dextran-coated ^{64}Cu-SPIONs, which clinicians are hoping to use for dual-mode MRI/PET imaging in the near future.[171]

Optical imaging has never reached its full potential in clinical practice and, for the most part, remains a preclinical/research imaging modality. Traditionally, optical imaging has relied on fluorescence but its in vivo applications have been limited by 1) the small number of fluorescent imaging agents available in the NIR spectrum, which limits the use of low-energy lasers to interrogate specimens; 2) high background autofluorescence from superficial tissues, which restricts the sensitivity and depth of this imaging modality; 3) the large spectral overlap between fluorescent imaging agents, which prevents the detection of multiple targets simultaneously; and 4) the rapid photobleaching of fluorescent molecules, which limits study duration.[172,173] A new class of nanoparticle that uses optical imaging is QDs. These are semiconductor nanocrystals typically made from selenides or sulfides of metals such as cadmium or zinc and range in size from 2 to 10 nm. The wavelength of the emitted light does not depend on the material of the QD, but rather its physical size. Hence, the ability to precisely control, or tune, the size of the QD determines the wavelength and color of the emitted light, otherwise known as the "size quantization effect." The QD emission profile can therefore be tuned to contain characteristic peaks at wavelengths across the visible spectrum independent of the excitation wavelength in order for the emitted light to be perceived by the human eye. QDs have also been shown to be approximately 20 times brighter and 100 times more stable (ie, less susceptible to photobleaching) than traditional fluorescent reporters, which allows them to have greater tissue penetration while also being more practical for long-term imaging.[174] To date, QDs have been used in a variety of molecular biology applications such as DNA detection, cell sorting and tracking, and targeting molecular markers in vivo.[175] Indeed, QDs have been bioconjugated with several

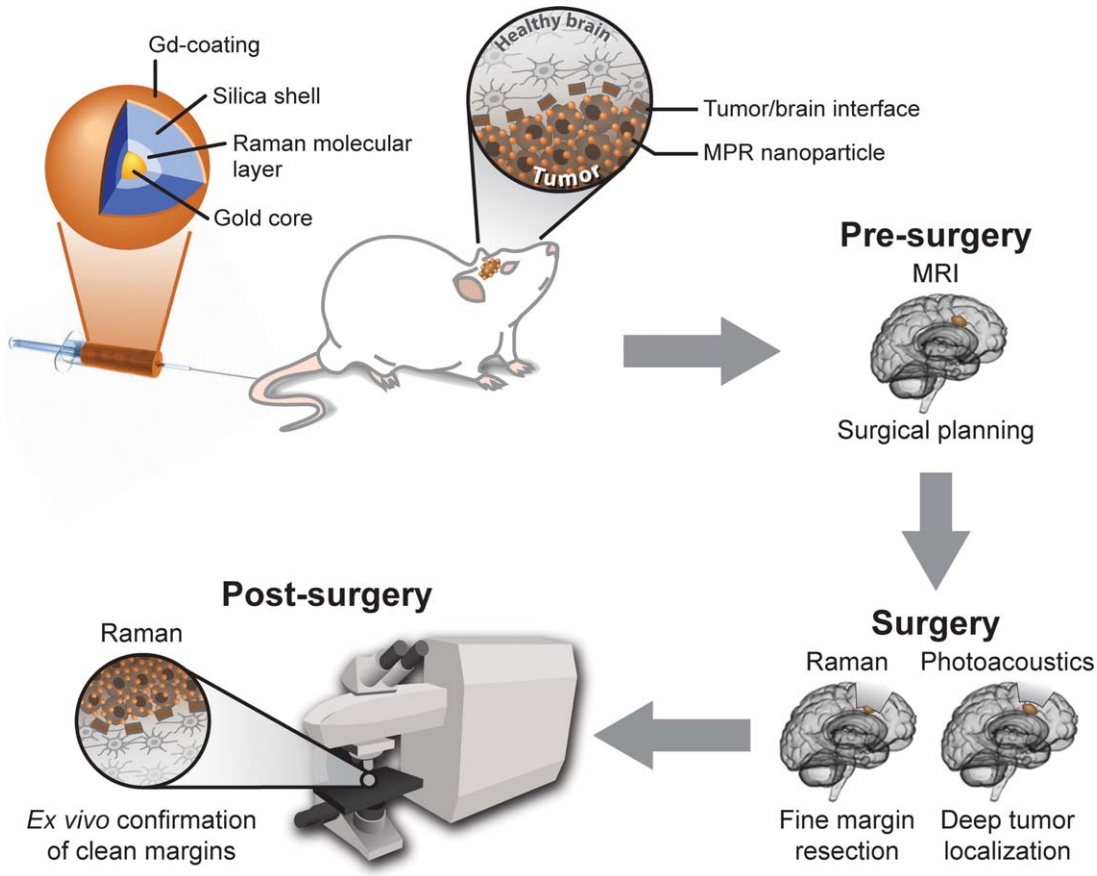

FIGURE 7. Multimodal Nanoparticles. Nanoparticles can be designed to be detected by more than one imaging modality (ie, magnetic resonance imaging [MRI], photoacoustic imaging, and Raman spectroscopy). When injected intravenously into mice with brain tumors, new triple-modality nanoparticles can accumulate within the tumor due to disruption in the blood-brain barrier. Preoperatively, their gadolinium (Gd) coating allows for their detection and hence tumor localization with MRI. During surgery, their Raman molecular layer guides fine tumor resection with Raman spectroscopy while their high optical absorbance coefficient allows photoacoustic imaging to guide deep tumor localization. After surgery, tissue specimens can be analyzed ex vivo using Raman spectroscopy to confirm tumor-free/clean resection margins. MPR indicates magnetic resonance-photoacoustic-Raman imaging.

ligands,[176] including with prostate-specific membrane antigen,[177] EGFR,[178] folate,[179] and RGD peptides[180] to name a few. Multimodality QDs are also being developed such as dual-function PET–near-infrared fluorescence (NIRF) QDs labeled with RDG peptides.[181] While the NIRF signal allows deeper tissue penetrance with fluorescence emissions beyond the spectral range of the signal produced by blood and tissues (ie, autofluorescence), thereby resulting in a high signal-to-background noise ratio, the PET signal allows highly quantitative tomographic imaging to be performed.

Another type of optical imaging known as Raman spectroscopy has also shown great promise in overcoming many of the limitations of fluorescence. In contrast to fluorescence, in which light is absorbed, Raman spectroscopy depends on the inelastic scattering of light. When monochromatic light, usually from a laser in the visible, NIR, or near-ultraviolet range impinges upon a molecule, some of the photons will bounce off, resulting in scattering. Occasionally, a portion of the energy of the incident photon is passed to the molecule causing it to vibrate, resulting in the photon being inelastically scattered with less energy. This energy exchange between scattering molecules and incident light is known as the "Raman effect." As the magnitude of the Raman effect is inherently weak (approximately 1 photon is inelastically scattered for every 10^7 elastically scattered photons), this limits the sensitivity and hence the clinical applications of Raman spectroscopy. Advances in nanotechnology have enabled the synthesis of nanoparticles that can overcome this problem by taking advantage of the phenomenon known as surface-enhanced Raman scattering (SERS). SERS is a plasmonic effect where molecules adsorbed onto a nano-roughened noble metal surface experience a dramatic increase in the incident electromagnetic field, resulting in high Raman signals.[182] Hence nanoparticles have been created with a 60-nm nano-roughened gold core coated with a monolayer of a "Raman organic molecule," which is encapsulated with a silica shell measuring 30 nm in diameter. This arrangement dramatically increases the incident electromagnetic field of the Raman organic molecule layer via SERS, thereby significantly amplifying the intensity of the Raman signal. This allows

for the detection of nanoparticles at picomolar concentrations in deep tissue, thereby making it ideal as an in vivo imaging agent. As the Raman organic molecule can be changed, each nanoparticle can carry its own spectral signature, thus allowing multiple nanoparticles to be independently detected simultaneously in vivo in a process known as multiplexing.[183] This is due to each Raman active layer having different chemical bonds resulting in different molecular vibrations after laser excitation, thereby giving them each their own unique Raman signature (Fig. 6). Recent research has shown that up to 5 spectral signatures can be identified and spectrally separated simultaneously in living subjects. Hence, if a specific signature is associated with a specific targeting ligand (ie, a peptide, monoclonal antibody, affibody, or aptamer) attached to the nanoparticle, a molecular profile of the cancer can be determined by spectrally separating the Raman signatures in the signal detected from a tumor. Recently, a unique triple-modality MRI-photoacoustic imaging-Raman imaging nanoparticle has been developed for detecting and assisting brain tumor resection (Fig. 7). The nanoparticle allows 1) whole-brain tumor localization for preoperative and intraoperative macroscopic delineation using MRI via its gadolinium coating; 2) high spatial resolution and 3-dimensional imaging using photoacoustic imaging via its gold core; and 3) high-sensitivity, high-specificity, and high-resolution surface imaging of intraoperative tumor resection margins using Raman imaging via its Raman organic layer.[184] Although the potential applications of SERS nanoparticles and QDs are tremendous, concerns regarding their potential toxicity (especially of the cadmium component) will first need to be addressed before we see their use in mainstream clinical practice.

Nanoparticles have also been extensively developed for photoacoustic imaging, which is a unique nonionizing imaging modality that synergizes optical and ultrasound imaging.[185] In this technique, nanosecond pulses of infrared light are absorbed and transformed into kinetic energy and localized heating, which in turn releases a pressure or RF wave that can be detected and translated into a real-time image in a similar fashion to ultrasound.[186] Optical absorption can be either associated with endogenous molecules (ie, hemoglobin) or achieved through externally administered molecules such as nanoparticles (ie, nanoclusters, SPIONs, gold nanoparticles, and single-walled carbon nanotubes [SWCNTs]). Nanoparticle imaging agents have been demonstrated to produce more photoacoustic signaling than small molecules on a mole-to-mole basis.[187-191] Although gold nanoparticles were initially favored due to their high absorption characteristics and ability to control their spectra (allowing for multiplexing),[192] their relatively large size results in rapid clearance from the circulation via the RES. Nonetheless, experimental studies using gold and copper nanoparticles have shown promising results with photoacoustic imaging for identifying sentinel lymph nodes when imaging the axilla for lymph node metastases from breast cancer.[186,193] However, the development of SWCNTs as a photoacoustic imaging agent has become of great interest due to their unique high aspect ratio (approximately 1:100) and high surface area-to-volume ratio. These attributes minimize their uptake by the RES while having an increased affinity for molecular targets due to their multivalency effects.[194] Indeed, SWCNTs have been conjugated with RGD peptides and used as a contrast agent for noninvasive photoacoustic imaging of tumor vasculature.[190] In addition, the high photoacoustic signal from SWCNTs allows for high-resolution, 3D photoacoustic images with substantial depth of penetration, precise depth information, and submillimeter resolution at nanomolar sensitivity, features that have not yet been achieved by other molecular imaging modalities. Newer studies are also examining magnetoacoustic imaging as a variation of photoacoustic imaging, in which MRI is used instead of infrared light to stimulate SPIONs at greater depths to create an ultrasound image.[195]

Nanoparticles as Theranostic Agents

Theranostics describes the ability of an agent, such as a nanoparticle, to be simultaneously used for diagnosis and treatment. The idea is to develop a smart nanoparticle that can diagnose, deliver targeted therapy, and monitor the response to therapy in a single integrated system.[82,196,197] By designing such multipurpose nanoparticles, it is hoped that drug development will be accelerated while costs and risks will be reduced. As polymerization and emulsifying techniques have improved, nanoparticles can now be created with hydrophilic and hydrophobic facets thereby allowing their loading with different active materials (ie, a hydrophilic contrast agent and a hydrophobic therapeutic agent and vice versa).

SPIONs used for MRI have been extensively studied as potential theranostic agents as they can be externally coated with a single chemotherapeutic drug (ie, methotrexate,[198] trastuzumab,[199] and temozolomide[200]), contain combined hydrophilic and hydrophobic chemotherapeutic drugs for improved therapeutic benefit in a double-emulsion capsule (ie, doxorubicin and paclitaxel[201]), or be loaded with chemotherapeutic drugs when developed as a hollow porous structure (ie, with cisplatin for controlled drug release).[202] SPIONs are also able to be cross-linked and combined with DNA, such as the p53 tumor suppressor gene, for use as an efficient gene delivery carrier that can be tracked with MRI.[203] More complex nanoplatforms are also being developed that use polymeric liposomal carriers that have a folate-coated PEGylated lipid shell for tumor

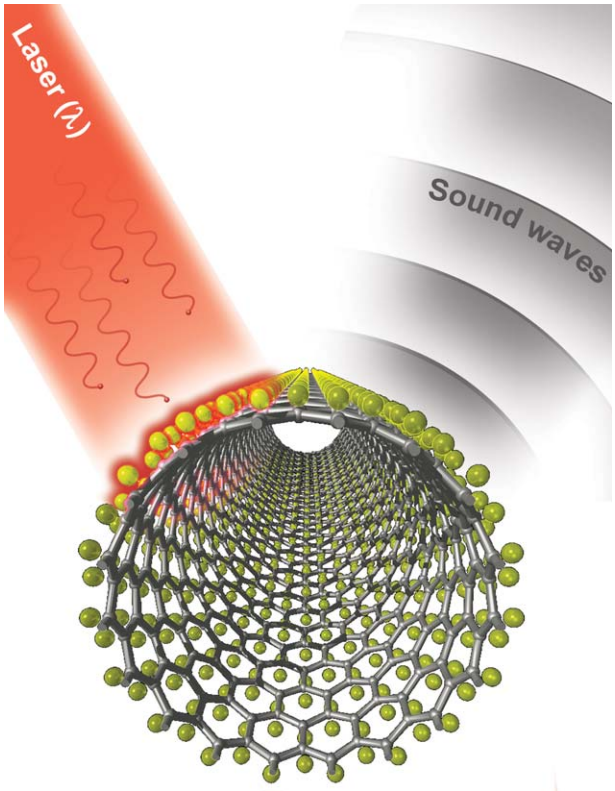

FIGURE 8. Theranostic Nanoparticles. Nanoparticles can be designed to be simultaneously used for diagnosis and treatment. Using near-infrared laser light, carbon nanotubes can be detected using photoacoustic imaging in addition to causing tumor cell thermal ablation via photothermal therapy.

targeting, which coencapsulates SPIONs for imaging and doxorubicin for controlled drug release.[204] Other platforms include using SPION cores with a polycationic surface coating (ie, poly(hexamethylene biguanide) or polyethyleneimine), which can bind siRNA through electrostatic interactions to form magnetic vectors that can be rapidly drawn to and concentrated on the surface of the target cells using the attractive force of an externally applied magnetic field. This facilitates the uptake of the magnetic vector into the cell endosomes, thereby improving siRNA transfection efficiency.[205] SPIONs have also been radiolabeled with ^{64}Cu (for combined imaging PET/MRI), conjugated with doxorubicin (for chemotherapy), and functionalized with RGD (for tumor vasculature targeting).[206] As PET has excellent sensitivity but relatively poor spatial resolution, its combination with MRI will provide excellent spatial resolution and soft-tissue contrast that is superior to CT while also not delivering any of ionizing radiation associated with CT to the patient. Furthermore, the conjugation of doxorubicin onto PEGylated SPIONS was performed via pH-sensitive hydrazone bonds, thereby allowing controlled drug release within the acidic microenvironment of tumors. Although these and many other elegant nanoplatform designs have been tested within cell culture, they have yet to be validated in living subjects. Nevertheless, these exciting results provide great promise for the future.

Carbon nanotubes (CNTs) have been studied for photoacoustic and optical imaging since they have a strong optical absorbance in the high-near infrared region of the electromagnetic spectrum (ie, 700-1100 nm), where biological systems have a transparent window.[207] This therefore makes them ideal for near-infrared photothermal ablation therapy, with the temperature within tumors shown to increase in a light-dependent and CNT dose-dependent manner (Fig. 8).[196,208] In addition, CNTs are being investigated for their use in gene and drug delivery, since they can readily cross biological barriers.[207] Although the mechanism by which CNTs are internalized by cells is not fully understood,[209] they can enter cells independently of cell type and surface functional groups. Due to the capacity of their backbone to form supramolecular complexes, CNTs have been conjugated with chemotherapeutic drugs such as doxorubicin,[210] methotrexate,[211] paclitaxel,[212] cisplatin,[213] and gemcitabine.[214] Several groups have also used CNTs for antitumor immunotherapy, whereby CNTs act as antigen-presenting carriers to improve weakly immunogenic tumor-based peptides/antigens to trigger a humeral immune response within the patient against the tumor.[215] Cationic CNTs have also been used as molecular transporters applicable for siRNA therapeutics to silence gene expression in both cell culture and in xenograft mice models.[216]

Gold nanoparticles that are used for optical and photoacoustic imaging can also be used in PTT. Following irradiation, the high electron density within the metallic lattice of gold nanoparticles results in absorption of photon energy that, in turn, causes the lattice and hence the nanoparticle to heat up. The small size and the rapid heating ability of gold nanoparticles are attractive for the selective heating and killing of cancer cells with an appropriate light source without the destruction of the surrounding normal and healthy tissue. Although NIR-mediated ablation has shown promise, its efficacy is limited by its depth of penetration, which only allows the treatment of superficial tumors up to 2 to 3 cm. However, RF ablation may be able to overcome this obstacle and allow the treatment of deep-seated tumors since gold nanoparticles have been shown to interact with shortwave RF waves to produce heat.[217] Currently, RF treatments use macroscopic electrodes to induce ablation, which is painful and can cause damage to surrounding tissues. However, the use of microelectrodes could make this technique less invasive and more effective provided that nanoparticles can be concentrated above a threshold level at the site of the tumor.[100] Multimodal nanoparticles have also been created such as those that have a superparamagnetic core to allow imaging with MRI, and a gold shell to allow PTT.[218]

Other constructs include microcapsules that are used for contrast-enhanced ultrasound imaging which are coated with a gold nanoshell to allow for PTT[219] and silica-coated gold nanorods that are used for PTT but also show strong x-ray attenuation for in vivo x-ray and CT imaging.[220] All these nanoparticles have the ability to be targeted to tumor cells once functionalized, thereby allowing focused and targeted antitumor therapy that can be closely monitored. In addition to PTT, gold nanoparticles are also being developed to deliver targeted therapeutics, such as drug and gene delivery (ie, DNA, RNA, and siRNA), to the site of tumors.[100] Gold nanoparticles can also be used as carriers of photosensitizer molecules to increase their aqueous solubility, bioavailability, stability, and delivery to target tissues. This is due to photosensitizers, such as phthalocyanine, being strongly hydrophobic and poorly water soluble, thereby limiting their therapeutic effectiveness.[221] In addition, gold nanoparticles can be used with photosensitizers for dual-modality treatment such as combined photodynamic therapy and PTT.[222]

Conclusions

This review has demonstrated many different applications for which nanoparticles are being used in the fight against cancer. Their unique attributes have allowed clinicians to offer them either as new treatments (monotherapy) or as adjuncts to existing treatments (combined therapy) to improve therapeutic effectiveness. Although some nanoparticles have not been successful when being clinically translated, several new and promising nanoparticles are currently in development and show great promise, thereby providing hope for new treatment options in the near future. However, all newly developed nanoparticles, whether they are used as carriers for drugs, therapeutic agents, or imaging agents, will need to be thoroughly characterized physiochemically, pharmacologically, and immunologically before they can be approved for use in humans. The distribution of nanoparticle size, uniformity, and consistency between batches also needs to be tightly regulated. Furthermore, for nanoparticles containing polymer layers and ligands, the loading density must be determined (ie, by using electron microscopy, electron dispersion spectroscopy, absorption spectroscopy, etc). Nanoparticles have been shown to possess very different properties compared with their corresponding bulk material, which has significant implications for their use in vivo since their small size will affect their mode of endocytosis, cellular trafficking, and processing. In addition, their high surface area-to-volume ratio, surface reactivity and charge will dramatically alter their chemical and physical properties, resulting in them possessing unexpected toxicities and biological interactions. Although several studies have investigated the toxicity associated with specific nanoparticles, the results are highly variable,[223–225] which can be attributed, in part, to the different shapes, sizes, and chemical preparations of nanoparticles as well as the type of human cell line studied. Hence, short-term and long-term toxicity studies will also need to be undertaken in both cell culture and living animal models before they can gain FDA approval for clinical trials. Nevertheless, with our continued drive to cure cancer and our determination to understand the molecular mechanisms that drive this disease to allow its early detection, nanotechnology provides hope in developing new ways to diagnose, treat, and follow patients with cancer in the 21st century. ■

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