Application of Nanotechnology in Cancer Therapy and Imaging

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ABSTRACT Recent developments in nanotechnology have provided researchers with new tools for cancer imaging and treatment. This technology has enabled the development of nanoscale devices that can be conjugated with several functional molecules simultaneously, including tumor-specific ligands, antibodies, anticancer drugs, and imaging probes. Since these nanodevices are 100 to 1,000-fold smaller than cancer cells, they can be easily transferred through leaky blood vessels and interact with targeted tumor-specific proteins both on the surface of and inside cancer cells. Therefore, their application as cancer cell-specific delivery vehicles will be a significant addition to the currently available armory for cancer therapeutics and imaging. (CA Cancer J Clin 2008;58:97–110.) © American Cancer Society, Inc., 2008.

INTRODUCTION Cancer is one of the major causes of mortality in the United States, and the worldwide incidence of cancer continues to increase. The most common cancer treatments are limited to chemotherapy, radiation, and surgery. Frequent challenges encountered by current cancer therapies include nonspecific systemic distribution of antitumor agents, inadequate drug concentrations reaching the tumor, and the limited ability to monitor therapeutic responses. Poor drug delivery to the target site leads to significant complications, such as multidrug resistance.

Greater targeting selectivity and better delivery efficiency are the 2 major goals in the development of therapeutic agents or imaging contrast formulations. Ideally, a therapeutic drug would be selectively enriched in the tumor lesions with minimal damage to normal tissues. A rational approach to achieve these goals is to conjugate therapeutic drugs with monoclonal antibodies (mAbs) or other ligands that selectively bind to antigens or receptors that are usually abundantly or uniquely expressed on the tumor cell surface. Several ligand-targeted therapeutic strategies, including immunotoxins, radioimmunoconjugates, and drug immunoconjugates, are being developed. Although these conjugated agents have demonstrated promising efficacy compared with conventional chemotherapy drugs in preclinical and clinical trials,1 limitations in their delivery efficiency and specificity remain. For example, in vivo studies have shown that only 1 to 10 parts per 100,000 of intravenously administered mAbs, therapeutic, or imaging agents can reach their parenchymal targets.2,3

At present, noninvasive imaging approaches, including x-ray–based computer-assisted tomography (CT), positron emission tomography (PET), single-photon emission tomography, and magnetic resonance imaging (MRI), are used as important tools for detection of human cancer.4–9 The development of tumor–targeted contrast agents based on a nanoparticle formulation may offer enhanced sensitivity and specificity for in vivo tumor imaging using currently available clinical imaging modalities.

By applying a vast and diverse array of nanoparticles, whose design derives from the engineering, chemistry, and medicine fields, to molecular imaging and targeted therapy, cancer nanotechnology promises solutions to several of the current obstacles facing cancer therapies. Nanoparticles have a mesoscopic size range of 5 to 200 nm, allowing their...
unique interaction with biological systems at the molecular level. As a result of their material composition, nanoparticles are capable of self-assembly and maintaining stability and specificity, which are crucial to drug encapsulation and bio-compatibility. Recent progress in cancer nanotechnology raises exciting opportunities for personalized oncology in which diagnosis and treatment are based on the molecular profiles of individual patients.

In this review, we will address first the types and characteristics of nanoparticles; second, how nanoparticles can be used as drug delivery systems and imaging devices to increase the efficacy per dose of therapeutic or imaging contrast agents; and last, how nanoparticles will be further developed to improve their functionality in cancer treatment and imaging.

NANOPARTICLES FOR TUMOR TARGETING AND DELIVERY

Types of Nanoparticles as Drug Delivery Systems

Nanoparticles can consist of a number of materials, including polymers, metals, and ceramics. Based on their manufacturing methods and materials used, these particles can adopt diverse shapes and sizes with distinct properties. Many types of nanoparticles are under various stages of development as drug delivery systems, including liposomes and other lipid-based carriers (such as lipid emulsions and lipid-drug complexes), polymer-drug conjugates, polymer microspheres, micelles, and various ligand-targeted products (such as immunoconjugates).10–13

Liposomes and Other Lipid-based Nanoparticles

Liposomes are self-assembling, spherical, closed colloidal structures composed of lipid bilayers that surround a central aqueous space. Liposomes are the most studied formulation of nanoparticle for drug delivery (Table 1). Several types of anticancer drugs have been developed as lipid-based systems by using a variety of preparation methods. Liposomal formulations have shown an ability to improve the pharmacokinetics and pharmacodynamics of associated drugs.1 To date, liposome-based formulations of several anticancer agents (Stealth liposomal doxorubicin [Doxil], liposomal doxorubicin [Myocet], and liposomal daunorubicin [DaunoXome]) have been approved for the treatment of metastatic breast cancer and Kaposi’s sarcoma.2,14,15,17,30–32

First generation liposomes have an unmodified phospholipid surface that can attract plasma proteins, which in turn trigger recognition and uptake of the liposomes by the mononuclear phagocytic system (MPS), which is synonymous with the reticuloendothelial system,1 resulting in their rapid clearance from the circulation. This property impedes the distribution of liposomes and their associated drug to solid tumors or other non-MPS sites of drug action. Second

| Compound                          | Name         | Status     | Indication                                      | References |
|-----------------------------------|--------------|------------|-------------------------------------------------|------------|
| Liposomal daunorubicin            | DaunoXome    | Market     | Kaposi’s sarcoma                                 | 14         |
| PEG-immunoliposome-doxorubicin    | MCC-465      | Phase I    | Various cancers, particularly stomach cancer    | 15, 16     |
| Stealth liposomal doxorubicin     | Doxil/Caelyx | Market     | Kaposi’s sarcoma; refractory ovarian cancer;    | 17, 18     |
|                                   |              |            | refractory breast cancer                         |            |
| Liposomal doxorubicin             | Myocet       | Market (Europe) | Metastatic breast cancer in combination with cyclophosphamide | 1, 17, 18 |
| Liposomal cisplatin               | SPI-077      | Phase I    | Various cancers                                 | 19–21      |
| Liposomal interleukin 2           | Oncolin      | Phase I    | Immune stimulant for use with a liposomal vaccine against non-small-cell lung cancer | 22         |
| Liposomal thymidylate synthase inhibitor | OSI-7904L | Phase II |                                                      | 23, 24     |
| Liposomal paclitaxel              | LEP ETU      | Phase I/II | Advanced solid tumors                           | 25         |
| Liposomal SN38 or liposomal irinotecan metabolite | LE-SN38 | Phase I/II | Advanced solid tumors                           | 26, 27     |
| Liposomal lurtotecan              | OSI-211      | Phase II   | Recurrent ovarian cancer; recurrent small cell-lung cancer | 28         |
| Liposomal oxaliplatin             | Aroplatin    | Phase II   | Advanced colorectal cancer                       | 29         |

Abbreviation: PEG, polyethylene glycol.
Generation liposomal drugs are being developed in an effort to evade MPS recognition and subsequent clearance. Surface-modified liposomes (Stealth) have hydrophilic carbohydrates or polymers, which usually are lipid derivatives of polyethylene glycol (PEG) grafted to the liposome surface.\(^1\)\(^{13}-^{15}\) While this surface modification has solved the problem of fast clearance from the circulation, yielding liposomes with a significantly increased half-life in the blood, the challenge remains to attain preferential accumulation of liposomes in tumor tissues. One strategy to achieve tumor-specific targeting is to conjugate a targeting moiety on the outer surface of the lipid bilayer of the liposome that selectively delivers drug to the desired site of action.\(^36-^{42}\) For example, an immunoliposome has antibodies or antibody fragments conjugated on its outer surface, usually at the terminus of PEG. Several studies have documented improved therapeutic efficacy of immunoliposomes targeted to internalizing antigens or receptors compared with that of nontargeted liposomes.\(^42-^{45}\) An in vitro study of a liposome formulation of doxorubicin (DOX) targeted to the internalizing antigen CD44 on B16F10 melanoma cells showed enhanced intracellular drug uptake from the targeted liposomes when compared with the free form of DOX. The enhanced uptake was correlated with enhanced cell killing efficacy.\(^46\)

**Polymeric Nanoparticles**

To reach the targeted tumor tissue, nanoparticles must be able to stay in the bloodstream for considerable lengths of time without being eliminated. Nanoparticles with no surface modification are usually caught by the MPS, primarily the liver and spleen, during circulation, depending on their size and surface characteristics.\(^11\) To overcome this problem, nanoparticles can be coated with hydrophilic polymers. Coating can efficiently protect nanoparticles from capture by macrophages.\(^48-^{50}\) The increased hydration also helps nanoparticles to be more water soluble and less sensitive to enzymatic degradation, therefore enhancing biocompatibility.\(^50,^{51}\)

During the past decade, the application of polymer-based drug delivery systems in oncology has grown exponentially with the advent of biodegradable polymers. In these polymers, drugs are either physically dissolved, entrapped, encapsulated, or covalently attached to the polymer matrix.\(^52\) The resulting compounds may have different structures, including micelles and dendrimers. Both natural (albumin, chitosan, heparin, etc.) and synthetic (poly-L-lactide, poly-[L-glutamate], poly-[D,L-lactide-co-glycolide], PEG, etc.) biodegradable polymers are being exploited as drug delivery systems.

Recently, a nanoparticle formulation of paclitaxel bound to albumin (Abraxane or ABI-007) was approved for the treatment of metastatic breast cancer.\(^53-^{55}\) In a Phase III clinical trial, ABI-007 showed greater therapeutic efficacy and increased response compared with free paclitaxel.\(^53,^{55}\) Currently, more than 10 formulations of anticancer polymeric nanoparticles have entered clinical development, including paclitaxel poliglumex (Xyotax),\(^56,^{57}\) N-(2-hydroxypropyl) methacrylamide (HPMA) copolymer-camptothecin (MAG-CPT),\(^58,^{59}\) and HPMA-DOX (PK1)\(^60\) (Table 2). In Phase I/II clinical trials, HPMA-DOX showed a 4 to 5-fold reduction in anthracycline-related toxicity.\(^63,^{64,^{76}}\) At DOX-equivalent doses of 80 to 320 mg/m², the drug still displayed significant antitumor activity in chemotherapy-refractory patients (including those with breast cancer).\(^76\) A recent Phase III trial showed that paclitaxel poliglumex (Xyotax) was less toxic than free paclitaxel and could prolong the survival of non-small-cell lung cancer patients with poor performance status.\(^61,^{77}\) Also, paclitaxel poliglumex can be used as a novel radiation sensitizer.\(^78\)

**Targeted Delivery of Therapeutic Nanoparticles**

In principal, nanoparticle delivery of anticancer drugs to tumor tissues can be achieved by either passive or active targeting.
Passive Targeting

Passive targeting takes advantage of the inherent size of nanoparticles and the unique properties of tumor vasculature, such as the enhanced permeability and retention (EPR) effect and the tumor microenvironment. This approach can effectively enhance drug bioavailability and efficacy.

**EPR Effect.** Angiogenesis is crucial to tumor progression. Angiogenic blood vessels in tumor tissues, unlike those in normal tissues, have gaps as large as 600 to 800 nm between adjacent endothelial cells. This defective vascular architecture coupled with poor lymphatic drainage induces the EPR effect, which allows nanoparticles to extravasate through these gaps into extravascular spaces and accumulate inside tumor tissues (Figure 1). Dramatic increases in tumor drug accumulation, usually of 10-fold or greater, can be achieved when a drug is delivered by a nanoparticle rather than as a free drug. However, the localization of nanoparticles within the tumor is not homogeneous. The factors that result in high concentrations of nanoparticles in one part of the tumor tissue but not in other parts are not well understood yet. In general, the accumulation of nanoparticles in tumors depends on factors including the size, surface characteristics, and circulation half-life of the nanoparticle and the degree of angiogenesis of the tumor. Usually, less nanoparticle accumulation is seen in pre-angiogenic or necrotic tumors.

**Tumor Microenvironment.** Hyperproliferative cancer cells have profound effects on their surrounding microenvironment. Tumors must adapt to use glycolysis (hypoxic metabolism) to obtain extra energy, resulting in an acidic microenvironment. In addition, cancer cells overexpress and release some enzymes that are crucial to tumor migration, invasion, and metastasis, including matrix metalloproteinases (MMPs). Tumor-activated prodrug therapy is an example of passive targeting that takes advantage of this characteristic of the tumor-associated microenvironment. A nanoparticle conjugating an albumin-bound form of DOX with an MMP-2–specific peptide...
sequence (Gly-Pro-Leu-Gly-Ile-Ala-Gly-Gln) was efficiently and specifically cleaved by MMP-2.90 When certain pH-sensitive molecules are incorporated into liposomes, drugs can be specifically released from the complexes by a change in pH.91 The pH-sensitive liposomes are stable at physiologic conditions (pH 7.2), but degraded in tumor-associated acidic areas. Likewise, thermodatable liposomes are expected to be activated by the local hyperthermic microenvironment.92

**Active Targeting**

The polymeric nanoparticles that have been tested clinically so far have mostly lacked a targeting moiety and instead rely mainly on the EPR effect of tumors, the tumor microenvironment, and tumor angiogenesis to promote some tumor-selective delivery of nanoparticles to tumor tissues. However, these drug delivery systems using a binary structure conjugate inevitably have intrinsic limitations to the degree of targeting specificity they can achieve. In the case of the EPR effect, while poor lymphatic drainage on the one hand helps the extravasated drugs to be enriched in the tumor interstitium, on the other hand, it induces drug outflow from the cells as a result of higher osmotic pressure in the interstitium, which eventually leads to drug redistribution in some portions of the cancer tissue.93

An alternative strategy to overcome these limitations is to conjugate a targeting ligand or an antibody to nanoparticles. By incorporating a targeting molecule that specifically binds an antigen or receptor that is either uniquely expressed or overexpressed on the tumor cell surface, the ligand-targeted approach is expected to selectively deliver drugs to tumor tissues with greater efficiency (Figure 2). Such targeted nanoparticles may constitute the next generation of polymeric nanoparticle drug delivery systems. Indeed, several targeted polymeric nanoparticles are currently undergoing preclinical studies.65,77,94–96 One of these, HPMA copolymer-DOX-galactosamine (PK2, FCE28069), has progressed to a clinical trial. In this nanoparticle, galactosamine moieties bind to the asialoglycoprotein receptor on hepatocytes.55,76 In a Phase I/II study, this targeted nanoparticle showed 12- to 50-fold greater accumulation than the free DOX in hepatocellular carcinoma tissue. Antitumor activity was observed in patients with primary hepatocellular carcinoma in this study.55,76 These promising early clinical results suggest the potential of targeted polymeric nanoparticles as anticancer treatment options.

**FIGURE 2** Internalization of Nanoparticles via Receptor-mediated Endocytosis. Nanoparticle-conjugated tumor-specific ligands/antibodies bind to surface receptors, triggering nanoparticle internalization through an endosome-dependent mechanism. As the interior of the endosome becomes more acidic, drugs are released from the nanoparticle into the cytoplasm.
drug delivery systems. Lessons have also been learned from many of the early clinical studies. For example, the failure of HPMA conjugates of paclitaxel and camptothecin in Phase I clinical trials was reported. Such negative outcomes underline the importance of polymer-drug design.66,97

**Choice of Target Receptor.** Selection of the appropriate receptor or antigen on cancer cells is crucial for the optimal design of targeted nanoparticles. The ideal targets are those that are abundantly and uniquely expressed on tumor cells, but have negligible or low expression on normal cells. The targeted antigen or receptor should also have a high density on the surface of the target tumor cells. Whether the targeted nanoconjugate can be internalized after binding to the target cell is another important criterion in the selection of proper targeting ligands. In the case of an antibody or other ligand that cannot trigger the internalization process, the drug can enter cells through simple diffusion or other transport system after being released from the targeted conjugate at or near the cell surface. However, drug released outside the cell may disperse or redistribute to the surrounding normal tissues rather than exclusively to the cancer cells. In vitro and in vivo comparisons using internalizing or non-internalizing ligands have shown that the intracellular concentration of drug is much higher when the drug is released from nanoparticles in the cytoplasm after internalization.43,98

**Choice of Targeting Ligand.** One of the greatest challenges to the design of nanoparticles that can selectively and successfully transport drug to cancerous tissues is the choice of targeting agent(s). This strategy also relies on the ability of the targeting agent or ligand to bind the tumor cell surface in an appropriate manner to trigger receptor-mediated endocytosis. The therapeutic agent will thereby be delivered to the interior of the cancer cell.89 A variety of tumor-targeting ligands, such as antibodies, growth factors, or cytokines, have been used to facilitate the uptake of carriers into target cells.90,92,99–107

Ligands targeting cell-surface receptors can be natural materials like folate and growth factors, which have the advantages of lower molecular weight and lower immunogenicity than antibodies. However, some ligands, such as folate that is supplied by food, show naturally high concentrations in the human body and may compete with the nanoparticle-conjugated ligand for binding to the receptor, effectively reducing the intracellular concentration of delivered drug. Recent advances in molecular biology and genetic engineering allow modified antibodies to be used as targeting moieties in an active-targeting approach. MAbs or antibody fragments (such as antigen-binding fragments or single-chain variable fragments) are the most frequently used ligands for targeted therapies. Whole mAbs have 2 binding domains showing high binding avidity. The Fc domain of the mAb can induce complement-mediated cytotoxicity and antibody-dependent, cell-mediated cytotoxicity, leading to additional cell-killing effect. On the other hand, the Fc domain also initiates an immune response and can be rapidly eliminated in the circulation, resulting in decreased accumulation of targeted nanoparticles into cancer cells.13 Compared with whole mAbs, the use of antibody fragments as a targeting moiety can reduce immunogenicity and improve the pharmacokinetic profiles of nanoparticles.1 For example, liposomes coupled with mAb fragments instead of whole antibodies showed decreased clearance rates and increased circulation half-lives, allowing the liposomes sufficient time to be distributed and bind to the targeted cells.1,39 This strategy improved the therapeutic efficacy of immunoliposomal DOX targeted against CD19 on human B lymphoma cells in animal models.1,39

**Reduction or Reversion of Multidrug Resistance**

Drug resistance is one of the major obstacles limiting the therapeutic efficacy of chemotherapeutic or biologic agents. In the clinic, chemoresistance is defined as either a lack of tumor-size reduction or the occurrence of clinical relapse after an initial positive response to antitumor treatment. Drug resistance can be caused by (1) physiological barriers (noncellular-based mechanisms) or (2) alterations in the biochemistry of cancer cells (cellular mechanisms). First, noncellular drug-resistance mechanisms can be due to physiological barriers, which protect cancerous cells from drug-induced cytotoxicity. One of the most effective barriers in the body is the
blood-brain barrier (BBB) that restricts the entry of anticancer agents to the brain from the periphery.\textsuperscript{108} The low permeability of the BBB is mainly attributed to microvessel endothelial cells in the brain. These cells contain an extremely active efflux pump system, similar to that identified in tumor cells, which removes a large volume of agents from the brain to the blood. A recent study showed that ulex europeus agglutinin I-conjugated nanoparticles can effectively bypass the BBB.\textsuperscript{109} The acidic environment in tumors can also result in resistance to basic drugs through neutralization. Further, high interstitial pressure and low microvascular pressure may also impede extravasation of drug molecules. Secondly, resistance of tumors to therapeutic intervention may be due to cellular mechanisms, such as altered activity of specific enzyme systems (for example, topoisomerase activity), altered apoptosis regulation, or increased drug efflux in malignant cells. Among these mechanisms, changes in the drug efflux pump are the best known and most extensively investigated. P-glycoprotein (p-gp), a product of the \textit{MDR1} gene, is a 170-kD transmembrane glycoprotein that functions as an efflux pump to remove drug out of cells, thus reducing the intracellular concentration of the drug. The p-gp pump usually recognizes substrate drugs and pumps them out of the cell as they pass through the plasma membrane. To date, several p-gp inhibitors have been investigated as potential anticancer agents. In preclinical studies, some of these p-gp inhibitors have shown the restoration of cancer-cell sensitivity to anticancer drugs. Unfortunately, when coadministered with anticancer agents, these inhibitors have generated considerable toxicity.\textsuperscript{110,111}

Given the enormous capacity of cancer cells to deploy various mechanisms to ensure their survival in the face of treatment with anticancer drugs, it is not surprising that promising strategies to inhibit drug resistance have proven difficult to translate into clinical success. Strategies for overcoming drug resistance should be based on new drug delivery systems, which will allow selective drug accumulation in tumor tissues, tumor cells, or even compartments of tumor cells. Nanoparticles are exemplars of such delivery systems, which aim to overcome both noncellular- and cellular-based drug resistance and to increase selectivity of drugs toward cancer cells while reducing their toxicity toward normal tissues.

By choosing an appropriate nanoparticle polymer carrier, it is possible to protect an antitumor drug from the acidic microenvironment it encounters before penetration into tumor cells. It is also expected that nanoparticles can bypass the p-gp efflux pump, leading to greater intracellular accumulation. For example, DOX-loaded poly (alkyl cyanoacrylate) nanoparticles were able to penetrate cells without being recognized by p-gp through forming an ion pair between degradation products and the drug.\textsuperscript{112} A clinical study from Northfelt’s group has shown that liposomal DOX is able to overcome drug resistance in AIDS-related Kaposi’s sarcoma.\textsuperscript{113,114} Another way to bypass the p-gp efflux pump is to deplete adenosine triphosphate (ATP), which is necessary for the ATP-dependent transporter to function properly. An ATPase inhibitor pluronic block copolymer (P85) has been shown to enhance the permeability of drugs through the BBB by inhibiting the p-gp drug efflux system.\textsuperscript{115,116} This effect was attributed to the combination of ATP depletion and ATPase inhibition. A micellar formulation of DOX using P85 has shown more effective apoptosis in drug-resistant breast cancer cells.\textsuperscript{115,116} Ligand-targeted strategies, especially those using receptor-targeting ligands, have also been applied to overcome drug resistance since these ligands are internalized via receptor-mediated endocytosis, bypassing the plasma membrane where p-gp primarily acts. As an example, folate receptor-targeted, pH-sensitive polymeric micelles containing DOX\textsuperscript{117} and transferrin-conjugated paclitaxel nanoparticles exhibited greater cytotoxicity than the respective free drugs in a drug-resistant model.\textsuperscript{118}

### MULTIFUNCTIONAL NANOPARTICLES FOR TUMOR IMAGING

Tumor imaging plays a key role in clinical oncology, with radiological examinations able to detect solid tumors, determine recurrence, and monitor therapeutic responses. Conventional tumor imaging approaches such as CT and MRI focus mainly on delineating morphological features of the tumor, tissue, and organs, such as the anatomic location, extent, and size of the tumor,
at various levels of spatial resolution and contrast. Despite continuous improvements in spatial resolution with advanced imaging equipment, imaging modalities using nontargeted contrast agents such as CT and MRI have limited sensitivity and ability to provide specific and functional information on the disease, which is increasingly recognized to be an obstacle to earlier diagnosis and the monitoring of treatment responses.

Recent advances have stimulated the emergence of the new field of “molecular imaging,” which focuses on visualizing or imaging biological events and processes in living systems, including patients. Current molecular imaging approaches, including PET, single-photon emission tomography, and optical imaging including fluorescence-mediated tomography and near-infrared fluorescence reflectance (NIRF) imaging, have shown a high sensitivity in non-invasive tumor imaging. A commonly used PET imaging probe, 18F-labeled fluorodeoxyglucose (FDG), can only localize tumors by identifying cells in the body that have increased glucose uptake and metabolism, allowing for the detection of those tumors. However, it is not suitable for tumor types with a low glucose uptake. It is well recognized that the development of novel approaches for early cancer detection and effective therapy will significantly contribute to the improvement of patient survival. The development of nanoparticles as imaging contrast agents also makes it possible for the production of multifunctional nanoparticles with the capacity of targeted tumor imaging and delivery of therapeutic agents. In comparison with radioactive probes (ie, 18F-labeled FDG) used for PET imaging, nanoparticles have both greater surface areas and more functional groups that can be linked with multiple diagnostic and therapeutic agents.

One molecular imaging strategy to improve the specificity of cancer detection is target-specific imaging of biomarker molecules specifically produced by cancer cells, coupled with imaging probes guided by ligands that can recognize and interact with target molecules. Recently, tumor-targeted optical, radioactive, or magnetic probes have been generated and their feasibility examined in animal tumor models and in very limited clinical studies. However, to develop this promising tumor imaging approach and eventually translate it to clinical applications, several important issues have to be addressed, including (1) identification of a target molecule that is highly expressed in most tumor cells, but is found at a low or undetectable level in normal cells; (2) production of stable and high-affinity targeting molecules in large enough quantities for potential in vivo imaging in animal models and eventual clinical use; (3) development of imaging probes emitting a strong enough signal to improve the sensitivity of cancer detection, but with a low toxicity to normal organs and tissues; and (4) increase in retention time of the targeted imaging probes in blood circulation, allowing for their accumulation to sufficient levels in the tumor mass.

Advances in nanotechnology have shown the promise of nanoparticles for tumor-targeted drug delivery and noninvasive tumor imaging. With unique pharmacokinetics, nanoparticles with sizes between 10 to 100 nm have a prolonged circulation time since they are usually not taken up by the MPS within the liver or excreted by the kidney, common limitations to the delivery of small molecular imaging agents or drugs. Such nanoparticles can navigate the vasculature and cross barriers through small capillaries into tumor cells. Extensive research has shown that nanoparticles in the above size range accumulate preferentially in tumor sites through the EPR effect associated with tumor growth. Moreover, the optical and electronic properties and biodistribution of many nanoparticles are often dependent on size. Nanoparticles of specific sizes can be synthesized under controlled conditions to obtain the desired optical and magnetic properties and levels of therapeutic agents attached to the particles. These properties offer the opportunity to design “smart” nanoparticles, including target-specific contrast agents, multimodality imaging probes, or even multifunctional reagents for simultaneous imaging and treatment.

Quantum Dot Nanoparticles

Semiconductor quantum dots (QDs) are nanometer-scale, light-emitting particles with unique optical and electronic properties such as size-tunable light emission, improved signal...
brightness, enhanced stability of the fluorescent signal, and the ability to simultaneously excite multiple fluorescent colors. These properties are most promising for improving the sensitivity of molecular imaging and quantitative cellular analysis by 1 to 2 orders of magnitude. Nie et al first reported that it is feasible to simultaneously target and image prostate tumors in living animal models using bioconjugated, prostate membrane antigen–targeted QDs. This new class of QD conjugate contains an amphiphilic triblock copolymer layer for in vivo protection and multiple PEG molecules for improved biocompatibility and circulation, making it highly stable and able to produce bright signals. Another advantage is that QD probes emitting at different wavelengths can be used together for imaging and tracking multiple tumor markers simultaneously, potentially increasing the specificity and sensitivity of cancer detection.

Recently, QDs producing NIRF signals have been developed. NIRF light penetrates much more deeply into tissues compared with visible fluorescence and allows for the detection of signals inside animals, as compared with visible fluorescent signals, which can only pass through several millimeters in the tissues (Figure 3). A major advantage of NIRF QDs is that their emission is well beyond the spectral range of the fluorescence signal produced by blood and tissues (autofluorescence), resulting in imaging with a high signal-to-background ratio. Detection of QD NIRF signals in sentinel lymph nodes within large animals in real time has been demonstrated. Therefore, QDs are excellent optical imaging nanoprobe for evaluating the specificity of tumor-targeting ligands in vitro in tumor cells and in vivo in animal tumor models. Sensitive real-time detection of tissue distribution of targeted QDs is also possible using the NIRF optical imaging system after systemic delivery. However, since cadmium is the main component of most QDs, there is some concern over their potential toxicity, making the feasibility of using these QDs for future clinical application still undetermined.

**Magnetic Iron Oxide Nanoparticles**

Superparamagnetic iron oxide (SPIO) or iron oxide (IO) nanoparticles are becoming increasingly attractive as the precursor for the development of a target-specific MRI contrast agent. IO nanoparticles have unique paramagnetic properties, which generate significant susceptibility effects resulting in strong T2 and T*2 contrast, as well as T1 effects at very low concentrations. In addition to the previously described unique properties and advantages of nanomaterials, IO nanoparticles have a long blood-retention time and are generally biodegradable and considered to have low toxicity. Several forms of IO nanoparticles have been used in clinical settings and have proven to be safe for human use. Some recent studies have demonstrated that IO nanoparticles can be internalized by various cell lines, which allows for magnetic labeling of the targeted cells. These features give IO nanoparticles great advantages for in vivo tumor imaging and drug delivery compared with other types of nanoparticles.

In recent years, significant efforts have been made to develop target-specific MRI contrast agents based on the formulation of IO nanoparticles. IO nanoparticles conjugated with ligands targeting cell surface markers such as MUC1, αvβ3 integrins, Her-2/Neu, or folate receptor have been reported. However, several obstacles remain to be overcome. One
of the major challenges is to develop a surface coating material that can not only stabilize the nanoparticle, but also provide active functional groups for controllable bioconjugation of "probe" ligands. For the specific needs of imaging applications in vivo, IO nanoparticles with a small size but high mass-magnetization value are desirable. For the specific purpose of cell targeting, activation of the particle surface for easy conjugation with biomolecules is essential. Because IO nanoparticles have low toxicity and a large surface area for carrying drugs, several studies have explored the feasibility of their use for the delivery of anticancer drugs. Indeed, the feasibility of simultaneous tumor MRI and drug delivery using \( \alpha \beta_3 \) integrin-targeted multifunctional polymeric micelles containing DOX and a cluster of SPIO nanoparticles has been demonstrated.\(^{145} \) In addition to chemotherapy drugs, IO nanoparticles for in vivo delivery of small interfering ribonucleic acids (siRNAs) have been developed in animal tumor models. SiRNAs can inhibit the expression of genes that are important for resistance to drug treatment by specifically binding to the target message RNAs, leading to their degradation. Such dual-purpose probes are capable of delivery of survivin siRNA, which targets an antiapoptotic protein that is upregulated in cancer cells, for cancer therapy as well as for simultaneous tumor imaging using MRI and NIRF imaging.\(^{126} \)

Recent efforts also focus on the development of ultrasensitive magnetic nanoparticles for tumor imaging. Using magnetism-engineered iron oxide nanoparticles that are conjugated with HER-2 antibodies, Lee et al showed an enhanced sensitivity of MRI for the detection of HER-2 expressing cancer in an animal model compared with that of commonly used SPIO probes.\(^{121} \) This new generation of magnetic nanoparticles should provide us with a powerful contrast agent for cancer detection.

**IMPLICATIONS AND FUTURE DIRECTIONS**

Cancer is known to develop via a multistep carcinogenesis process and to progress using several complex survival mechanisms, such as self-sufficiency in growth signaling, insensitivity to growth inhibitory signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, and tumor invasion and metastasis.\(^{146} \) To date, cancer treatments are performed on the basis of clinical and pathologic staging that is determined using morphologic diagnostic tools, such as conventional radiological and histopathological examinations. However, even patients suffering from cancers of the same cellular type and clinical stage respond to the same conventional treatment modalities differently and, ultimately, with variations in survival rate. This implies that cancer-associated events are unique in each patient.

Recent advances in molecular, biological, and genetic diagnostic techniques have begun to explore cancer-associated biomarkers and their implication for the development and progression of cancer and to reveal that cancer is controlled by complex multifactorial mechanisms rather than a single factor.\(^{147} \) Molecularly targeted therapy is a recent introduction acknowledging our increased understanding of these cancer behaviors at the molecular level. Success of targeted therapies depends on expression of the targeted molecules, which can also serve as cancer-specific biomarkers.\(^{148} \) Assays to accurately and quickly quantify several cancer-related biomarkers simultaneously on single tumor sections or small tumor specimens will be enabled by virtue of advances in nanotechnology.\(^{149,150} \) For example, the use of conjugated QDs potentially allows 5 cancer-related proteins to be detected on the same tissue section.\(^{151} \)
In addition to ex vivo analysis for the detection of early cancer and profiling of molecular biomarkers, in vivo imaging of cancer using several types of nanoparticles has also been investigated together with the progression of nanoscale drug delivery systems. The development of multifunctional nanoparticles may contribute significantly to the realization of individualized therapy for cancer. Ideally, for constructing multifunctional nanoparticles, an appropriate combination of agents (therapeutic agent and targeting moiety) will be chosen based on accurate biological information within the tumor (molecular biomarker profiling of the patient) with imaging material attached on the nanoparticle surface (Figure 4). Nanoparticles may eventually be capable of detecting malignant cells (active-targeting moiety), pinpointing and visualizing their location in the body (real-time in vivo imaging), killing the cancer cells with minimal side effects by sparing normal cells (active targeting and controlled drug-releasing system), and reporting back that their payload has accomplished its mission (monitoring treatment effects in real time).

Several kinds of nanoparticles have been evaluated to identify their potential as multifunctional nanoparticles that can be applied for simultaneous in vivo imaging and treatment of cancers. For example, 131I-labeled fluorescein isothiocyanate-conjugated glycol chitosan nanoparticles loaded with DOX exhibited selective localization in tumor tissues, resulting in clear delineation of tumor tissue against adjacent normal tissues by radionuclide imaging. This particle was suggested to be used as a potential carrier to direct the drug to tumor tissues. A type of magnetic nanocrystals, which consisted of FeCo in the core and surrounding graphitic shell, displayed long-circulating positive contrast enhancement by MRI in an in vivo animal model. It also significantly increased temperature under near-infrared laser radiation, suggesting a potential application in simultaneous imaging and photo-thermal ablation therapy. A recent study of a targeted multifunctional nanoparticle for imaging and photodynamic therapy showed a significantly improved therapeutic efficacy when compared with a nontargeted nanoparticle in an animal model. Since this nanoparticle formulation consisted of an encapsulated imaging agent and photosensitizer, treatment effects can be reported in real time by using MRI imaging.

With this promising progress in the development of nanotherapeutic and imaging approaches to cancer detection and treatment, it is imperative to have a better understanding of the basic principles involved in designing and applying nanoparticles for diagnosis, treatment, or the combination of imaging and therapeutics in different clinical situations. There are certain critical questions that need to be addressed in the rational design and application of nanoparticles before further clinical development, such as how the association or conjugation of a therapeutic agent to ligand or carrier changes the pharmacokinetics, biodistribution, and side effects of the nanotherapeutic drugs; how the safety profile of nanoparticles changed after conjugation, such as coating with QDs; how we can minimize the potential toxicity of polymeric nanoparticles that is inherent from the accumulation of a non-biodegradable polymer with a size over the renal threshold; and how side effects resulting from the ability of nanoparticles to cross the BBB can be prevented or diminished. These questions are critically important and hitherto understudied. The answers will certainly lead to more rational design of optimized nanoparticles with improved selectivity, efficacy, and safety. Attracted by the rapid and promising progress in nanotechnology, physicists, chemists, engineers, biologists, and clinicians will continue to challenge themselves to develop novel and efficacious nanosystems for the diagnosis and treatment of cancer.

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