Targeted magnetic iron oxide nanoparticles for tumor imaging and therapy

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Abstract: Magnetic iron oxide (IO) nanoparticles with a long blood retention time, biodegradability and low toxicity have emerged as one of the primary nanomaterials for biomedical applications in vitro and in vivo. IO nanoparticles have a large surface area and can be engineered to provide a large number of functional groups for cross-linking to tumor-targeting ligands such as monoclonal antibodies, peptides, or small molecules for diagnostic imaging or delivery of therapeutic agents. IO nanoparticles possess unique paramagnetic properties, which generate significant susceptibility effects resulting in strong T 2 and T* 2 contrast, as well as T 1 effects at very low concentrations for magnetic resonance imaging (MRI), which is widely used for clinical oncology imaging. We review recent advances in the development of targeted IO nanoparticles for tumor imaging and therapy.

Keywords: iron oxide nanoparticles, tumor imaging, MRI, therapy

Introduction

Cancer remains one of the leading causes of death in the world. Despite advances in our understanding of molecular and cancer biology, discovery of cancer biomarkers and conventional surgical procedures, radiotherapy, and chemotherapy, the overall survival rate from cancer has not significantly improved in the past two decades (Jemal et al 2008). The development of novel approaches for early detection and cancer marker-specific and personalized treatment of cancers is urgently needed to increase patient survival.

Recent advances in nanoscience and nanotechnology have led to the development of nanomaterials for molecular and cellular imaging, cancer therapy, and integrated nanodevices for cancer detection and screening (Jain 2005; Nie et al 2007; Sengupta and Sasisekharan 2007; Wang et al 2007). It is highly desirable that nanoparticles can not only provide sensitive and specific imaging information in cancer patients but also selectively deliver anticancer drugs to tumor sites. Currently, there is limited knowledge of suitable biomarkers for imaging, selection of the imaging target and contrast enhance materials, and the chemistry required to assemble the bioactive imaging probe. In addition, numerous obstacles are faced in developing cancer-specific imaging agents, such as 1) delivery of the probe to the targeted tissue/tumor; 2) biocompatibility and toxicity; 3) stability of the probe and effective signal enhancement in vivo; 4) adequate imaging methods and strategies. During chemotherapy, pharmacologically active cancer drugs reach the tumor tissue with poor specificity and induce dose-limiting toxicities. Nanoparticle drug delivery may provide a more efficient, less harmful solution to overcome these problems.

To date, the development of tumor-targeted nanoparticles remains extremely challenging. Magnetic resonance imaging (MRI) provides superb image resolution and exquisite soft tissue contrast for revealing tissue morphology and anatomical details, while allowing for whole body imaging of animals and humans. Although MRI has
become one of the primary oncology imaging modalities, its sensitivity is challenged when applied to molecular and cellular imaging (Bradbury and Hricak 2005; Ito 2006). To obtain contrast enhancement and signal amplification, magnetic contrast agents are often used. Although gadolinium diethylenetriaminopentaacetic acid (Gd-DTPA), which shows a strong T1 shortening effect, is widely accepted in clinical use, it has relatively low contrast effects and a very short retention time in vivo. In addition, the toxicity and biocompatibility of gadolinium during and after endocytosis by cells are still largely unknown (Bird et al 1988; Bulte and Kraitchman 2004; Kim et al 2007). Recently, magnetic iron oxide (IO) nanoparticles have emerged as a new generation of target-specific MRI T2 contrast agents. Magnetic IO nanoparticles are much more efficient than Gd-DTPA as relaxation promoters and their magnetic properties can be manipulated by controlling the sizes of core and coating surface (Rogers and Basu 2005). More importantly, IO nanoparticles have a long blood retention time, biodegradability and low toxicity (Harisinghani et al 2003; Funovics et al 2004; Jain et al 2005; Bradbury and Hricak 2005; Montet et al 2006). In this review, we focus on recent advances in the development of targeted IO nanoparticles for tumor imaging and therapy.

Production of magnetic iron oxide nanoparticles and functionalization of the nanoparticle surface

There are many different kinds of chemical methods for synthesizing magnetic nanoparticles. The most commonly used are precipitation-based approaches, either by coprecipitation or reverse micelle synthesis (Shen et al 1993; Nitin et al 2004). IO nanoparticles without any surface coating are not stable in aqueous media and readily aggregate and precipitate. For in vivo applications, the particles often form aggregates in blood and are sequestered by macrophages (Lee et al 2006). Therefore, the surface of IO nanoparticles should be coated with a variety of different moieties that can eliminate or minimize their aggregation under physiological conditions. Usually, two main approaches are used for coating magnetic IO nanoparticles, including in situ coatings with which the magnetic nanoparticles are coated during the synthesis process and post-synthesis coatings (Berry et al 2004; Jodin et al 2006; Horak et al 2007). In addition, magnetic IO nanoparticles can also be encapsulated in liposomes to create magnetoliposomes (De Cuyper and Joniau 1988).

The amphiphilic polymeric surfactants such as poloxamers, poloxamines and poly(ethylene glycol) (PEG) derivatives are usually used for coating the surface of IO nanoparticles, since they can minimize or eliminate opsonization of IO nanoparticles. Among them, PEG is the most used chemical material, which confers on IO nanoparticles several important properties such as high solubility and stability in aqueous solutions, biocompatibility, and prolonged blood circulation time. More importantly, the functional groups of modified PEG allow for bioconjugation of various ligands or therapeutic agents to IO nanoparticles (Kohler et al 2004; Mikhaylova et al 2004; Nitin et al 2004; Gupta and Gupta 2005; Veiseh et al 2005; Lee et al 2006, 2007a; Kumagai et al 2007). However, PEG-coated IO nanoparticles may have limited binding sites available for further ligand binding, since the number of functional groups on the surface of each IO nanoparticle is limited (Gupta and Gupta 2005). Lacoste and colleagues (2007) reported that the molecular weight of the PEG portion of the micelle coating is related to the overall IO nanoparticle diameter, while coating thickness can significantly affect their relaxivity. Our group recently observed that the molecular weight of PEG could significantly affect the distribution of PEG-coated IO nanoparticles in vivo. Thus, it is critical to select the ratio and molecular weight of PEG when designing IO nanoparticle probes for targeted imaging and therapy in vivo.

In addition to PEG coating, other materials such as antibiofouling poly(TMSMA-r-PEGMA) (Lee et al 2006), hyaluronic acid (HA) layers (Kumar et al 2007) and carboxyl-functionalized poly(amidoamine) (PAMAM) dendrimers of generation 3 (G3) (Shi et al 2007) have also been used to coat the surface of IO nanoparticles for either increasing circulation time in the blood or delivering peptides at high efficiency.

Recently, we have developed a new class of superparamagnetic iron particles that have uniform sizes ranging from 5–30 nm and can be further functionalized through surface coating with amphiphilic triblock polymers, which provide functional groups for conjugating tumor-targeting biomolecules such as peptides or antibodies. The triblock polymer developed in our group has surface reactivity for introducing various or multiple functional groups including the carboxylate group that can be used to cross-link “probe molecules” for biomarker-targeted specific binding (Gao et al 2004).

Despite significant efforts in developing MRI contrast agents based on IO nanoparticle formulations, several obstacles remain to be overcome. The major challenge 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. Traditional
Iron oxide nanoparticles for tumor imaging and therapy

The use of magnetic nanoparticles in medicine is rapidly evolving. Iron oxide (IO) nanoparticles have emerged as a versatile platform for a variety of biomedical applications because of their high magnetic moment and tunable size. IO nanoparticles can be used for magnetic resonance imaging (MRI) and targeted drug delivery. Moreover, IO nanoparticles can be functionalized with ligands such as antibodies or peptides to achieve targeted delivery. This review outlines recent advances in the use of IO nanoparticles for tumor imaging and therapy.

Targeted IO nanoparticles for tumor imaging

Although the feasibility of using IO nanoparticles for cancer detection and drug delivery has been demonstrated (Corot et al. 2006; Thorek et al. 2006), a major obstacle limiting their clinical application is that nontumor-targeted nanoparticles are unable to reach sufficient concentrations in the tumor site to either produce a strong signal for tumor imaging or to carry optimal amounts of therapeutic agents into tumor cells. One approach to overcome this problem is to develop tumor-targeted IO nanoparticles that are highly sensitive imaging probes and/or are capable of conjugating large amounts of therapeutic agents. This can be achieved by engineering the surface of the nanoparticles to recognize specific tumor-associated antigens using antibodies or peptides.

Recent advances in targeting tumor-associated antigens using IO nanoparticles include the conjugation of IO nanoparticles with monoclonal antibodies or small peptides. Antibody-based targeting has been shown to significantly increase the specificity and efficacy of IO nanoparticles. However, the size of antibodies used in these studies is very large and is not ideal for efficient conjugation to the surface of IO nanoparticles. To solve this problem, small peptides with a molecular weight of 1,000 Da or less can be used as target moieties for engineered targeted IO nanoparticles. These peptides can be internalized via receptor-mediated endocytosis, which allows for the delivery of therapeutic agents to the tumor site.

Figure 1: Targeted IO nanoparticles for tumor imaging in vivo. The tumor-specific ligands/antibodies were conjugated to the surface of SPIO coated by PEG. Targeted IO nanoparticles accumulate in solid tumor tissue mainly by receptor-mediated endocytosis and are usually taken up by macrophages in the liver (Kupffer cells) and spleen.

In conclusion, the targeted delivery of IO nanoparticles is a promising approach for cancer therapy and imaging. The development of novel targeting molecules, such as small peptides, can further improve the specificity and efficiency of targeted IO nanoparticles.

References

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Remsen et al. 1996
Tiefenauer et al. 1996
Artemov et al. 2003
Funovics et al. 2004
Huh et al. 2005
Toma et al. 2005
Serda et al. 2007
Lee et al. 2007b
Table 1 Targeted iron oxide nanoparticles for tumor imaging

| Iron oxide nanoparticles | Targeting ligands | Targets | Tumor | Experimental conditions |
|--------------------------|------------------|---------|-------|-------------------------|
| USPIO (Cerdan et al 1989) | Monoclonal antibody-610 | Surface antigen | Colon carcinoma cell lines | *In vitro* |
| SPIO (Tiefenauer et al 1996) | Antibody to carcinoembryonic antigen (CEA) | CEA | Colon tumor | *In vivo* |
| MINO (Remsen et al 1996) | Monoclonal antibody L6 | Surface antigen | Intracranial tumor LX-1 | *In vivo* |
| USPIO (Kresse et al 1998) | Transferrin | Transferrin receptor | Rat mammary carcinoma | *In vivo* |
| Streptavidin-conjugated SPIO (Artemov et al 2003) | Monoclonal antibody-Her/Neu | Her-2/neu receptors | Breast cancer | *In vitro* |
| CLIO-NH2 (Moore et al 2004) | EPPT peptide | Underglycosylated mucin-1 antigen (uMUC-1) | Breast, colon, pancreas and lung cancer cell lines | *In vivo* |
| Dextran-coated super-paramagnetic maghemite (γ-Fe₂O₃) nanocrystals (Sonvico et al 2005) | Folic acid | Folate receptor | Human epithelial mouth carcinoma | *In vitro* |
| Ferumoxides (SPIO) (Toma et al 2005) | Monoclonal antibody A7 | Colorectal tumor antigen | Colorectal carcinoma | *In vivo* |
| Iron oxide nanocrystals (Fe₂O₃) (Huh et al 2005) | Herceptin | Her-2/neu receptors | NIH3T6.7 | *In vivo* |
| SPIO (Kohler et al 2005) | Methotrexate | Folate receptor | Human cervical cancer cells | *In vitro* |
| SPIO (Veiseh et al 2005) | Chlorotoxin peptide | Membrane-bound matrixmetalloproteinase-2 (MMP-2) | Rat glioma | *In vitro* |
| Biofunctional PEG-SPIO (Sun et al 2006) | Folic acid | Folate receptor | Human cervical cancer cells | *In vitro* |
| SPIO encapsulated with photodynamic agent (Reddy et al 2006) | F3 peptide | Surface-localized tumor vasculature | Rat glioma | *In vivo* |
| HFn-IO (Uchida et al 2006) | RGD4C | α5β3 integrins | Melanoma cells | *In vitro* |
| SPIO (Leuschner et al 2006) | Luteinizing hormone releasing hormone (LHRR) | LHRH receptor | Breast cancer | *In vivo* |
| SPIO (Simberg et al 2007) | CREKA peptide | Clotted plasma proteins | Breast cancer | *In vivo* |
| USPIO (Zhang et al 2007) | Arg-Gly-Asp (RGD) | α5β3 integrins | Human epidermoid carcinoma | *In vivo* |
| PEG-SPIO (Chen et al 2007b) | Folic acid | Folate receptor | Human epithelial mouth carcinoma | *In vivo* |
| Streptavidin-SPIO (Serdia et al 2007) | Antibody to Prostate-specific membrane antigen (PSMA) | PSMA | Human prostate cancer cells | *In vitro* |
| Magnetism-engineered iron oxide (MEIO) nanoparticles (Lee et al 2007b) | Herceptin | Her-2/neu receptors | NIH3T6.7 | *In vivo* |
| PEG-IO (Sun et al 2008) | Chlorotoxin | Membrane-bound matrixmetalloproteinase-2 (MMP-2) | Rat glioma | *In vivo* |
will increase the uptake of conjugated IO nanoparticles and provide persistent MRI contrast enhancement, therefore, such types of peptides are ideal ligands for constructing targeted IO nanoparticles for tumor imaging. Chlorotoxin (Cltx) is a 36-amino acid peptide that can specifically bind to matrix metalloproteinase-2 (MMP-2) on the surface of cells. MMP-2 is overexpressed in gliomas and other related cancers and degrades the extracellular matrix during cancer invasion (Soroceanu et al 1998; Deshane et al 2003; Veiseh et al 2007). Sun and colleagues (2008) conjugated Cltx to IO nanoparticles with covalently bound bifunctional PEG polymer and showed that internalization of the Cltx-conjugated IO nanoparticles by 9L glioma cells was 10-fold higher than that of the nontargeted nanoparticles after 2hrs incubation. The R2(1/T2) relaxivity was 5.20mm⁻¹s⁻¹ and 0.22mm⁻¹s⁻¹ for the tumor cells after incubation with the Cltx-targeted IO nanoparticles and nontargeted IO nanoparticles, respectively. In vivo MRI showed that the tumor contrast enhancement in the superimposed R2 change was significantly higher in the mouse injected with Cltx-targeted IO nanoparticles than in the mouse receiving the nontargeted nanoparticles (Sun et al 2008).

The development of targeted IO nanoparticles for early tumor detection remains challenging. Underglycosylated mucin-1 antigen (uMUC-1) is an early tumor marker that is overexpressed on almost all human epithelial cell adenocarcinomas. Some important features render uMUC-1 a promising target for tumor imaging, 1) expressed in over 50% of all human cancers and remained homogeneously upregulated during the life growth of the tumor, 2) underglycosylated in tumor tissues but heavily glycosylated in normal tissues, make it possible to design probes that discriminate between normal and adenocarcinoma cells, 3) ubiquitously expressed on the cell surface, making it an accessible target for binding and imaging. Moore and colleagues (2004) synthesized EPPT1 peptide which specifically recognizes uMUC-1 and conjugated it to the dextran coat of crosslinked superparamagnetic iron oxide nanoparticles (CLIO). As shown in Figure 2, 24 hours after injection of targeted CLIO nanoparticles, a significant T2 signal reduction was observed in some regions of uMUC-1-positive LS174T tumors, while no significant change was seen in uMUC-1-negative U87 tumors. In addition, these results were further demonstrated by near-infrared fluorescence (NIRF) imaging. In this study, NIRF Cy5.5 dye-labeled CLIO nanoparticles were used both as MR- and NIRF-imaging contrast agent. This unique imaging probe produced a high-resolution signal on MR images and real-time NIRF imaging data, providing comprehensive information on tumor localization, environment, and status. This agent may have the potential to be applied for early tumor detection (Moore et al 2004). To date, tumor metastasis is still one of the main causes of death for breast cancer patients. Approximately 37% of breast cancer patients have tumor metastases in the bone and lymph nodes at the time of diagnosis, and the 5-year survival rates for these patients is only 27% (Jemal et al 2008). Development of targeted IO nanoparticles that could be used for the detection of early metastasis may improve the 5-year survival rates of breast cancer patients. About 52% of human breast cancers express binding sites for receptors for luteinizing hormone-releasing hormone (LHHRH) (Chatzistamou et al 2000). LHHRH is a decapeptide that has the primary sequence of EHWSYGRLPG. LHHRH-SPIO nanoparticles specifically accumulated in primary tumor cells and metastatic cells through receptor-mediated endocytosis, and the concentration of targeted SPIO nanoparticles was 12-fold higher than that of SPIO nanoparticles in vitro. In vivo data showed that the expression of LHHRH-SPIO nanoparticles was 7.5-fold higher in tumors and 11-fold higher in lung metastatic cells than that of nontargeted nanoparticles. After conjugating LHHRH to SPIO nanoparticles, in addition to receptor targeting, LHHRH may render the nanoparticles neutral, further increasing their circulation time and decreasing their recognition by the RES in vivo. This study demonstrated that LHHRH-conjugated SPIO nanoparticles could be used as an MRI contrast agent to detect metastatic breast cancer cells in vivo with high sensitivity (Leuschner et al 2006). One of the interesting results was that LHHRH-SPIO nanoparticles were found by TEM study to accumulate in the cytosol and the nucleus in the breast cancer cells; this may be an advantage for delivering drug in the future, since it seems this unique targeted IO nanoparticle could escape from the endosome.

Angiogenesis plays a critical role in the development of tumors; the α,β3 integrin is a marker of angiogenesis and its expression correlates with tumor grade. Therefore, α,β3 integrin is an ideal target for in vivo tumor imaging since the target is present on the surface of the vessels and can directly be accessed from the blood. Zhang and colleagues (2007) used 3-aminopropyltrimethoxysilane (APTMS) with functional amino groups as a coating material for modification of IO nanoparticles. APTMS can form a very thin monolayer on the surface and can be used to covalently attach related ligands. The Arg-Gly-Asp (RGD) peptide which binds to the α,β3 integrin receptor was conjugated to APTMS-coated USPIO nanoparticles. Following systemic administration of the RGD-USPIO nanoparticles in nude mice bearing tumors with different levels of α,β3 integrin-positive vessels, results
showed that RGD-USPIO nanoparticles targeted to the tumor vessels and the change in T2 relaxation was related to the degree of expression of \( \alpha \beta_3 \) integrin detected by 1.5-T MR scanner (Zhang et al 2007).

To increase the sensitivity of \textit{in vivo} tumor imaging of nanoparticles, it is necessary to deliver large amounts of the nanoparticles not only into the tumor cells but also to a tumor mass. Most of the currently used target molecules, such as Her-2/neu, \( \alpha \beta_3 \) integrin, PMSA and MUC-1, are expressed in subpopulations of tumor tissues or specific tumor types. Recently, Simberg and colleagues (2007) synthesized the tumor-homing peptide CREKA (Cys-Arg-Glu-Lys-Ala), which can form a distinct meshwork in the tumor stroma specifically. A CREKA-conjugated nanoparticle accumulated in both tumor vessels and stroma, resulting in intravascular clotting in tumor blood vessels which attracted more nanoparticles into the tumor, amplifying the targeting. There are several advantages of such targeted-SPIO nanoparticles, 1) high specificity for tumor homing, 2) enhanced MR imaging in tumor, 3) physical blockade of tumor vessels by local embolism. The clotting caused by CREKA-SPIO nanoparticles in tumor vessels may improve tumor detection by optical imaging techniques. Another potential application of the nanoparticle is for constructing drug delivery nanoparticles which can deliver drugs in tumor vessels and slowly release them (Simberg et al 2007).

The low molecular weight vitamin folic acid (FA), whose receptor is overexpressed on the surface of many human tumor cells, has been studied as a targeting agent. The advantages of using FA as a targeting ligand for tumor imaging include: 1) relatively higher binding affinity for its receptor (KD = 10^{-10} M), 2) low cost, easy conjugation with both therapeutic and imaging agents, 3) compatibility in both organic and aqueous solvents, 4) lack of immunogenicity (Low et al 2008). Sun and colleagues (2006) used heterobifunctional PEG 600 to coat the surface of IO nanoparticles and subsequently attached FA to the nanoparticles through an amide linkage at the free terminus of PEG. Their results showed that folate receptor-positive human cervical carcinoma HeLa cells took up about 12-fold more FA-IO nanoparticles than nontargeted IO nanoparticles (Sun et al 2006). One recent study showed by MRI that SPIO-PEG-FA could target human nasopharyngeal epidermoid carcinoma (KB) cells both \textit{in vitro} and \textit{in vivo} (Chen et al 2007).

\textit{In vivo} tumor imaging with MRI requires the delivery of sufficient concentrations of IO nanoparticles. Pinkernelle and colleagues (2005) reported that single IO nanoparticle-labeled human colon carcinoma cells can be detected using MRI techniques in vitro, the lowest concentration of iron needed is about 4–5 \( \mu g/10^6 \) cells (Pinkernelle et al 2005). For imaging by targeted IO nanoparticles, the sensitivity depends on the target concentrations in tumor cells, for example, some targets are often quite weakly expressed (10^4 folate receptors in brain glioma cells) (Saul et al 2003) while others are very highly expressed (3 \times 10^6 epidermal growth factor receptors in A431 human squamous carcinoma cells) (Jinno et al 1996). In addition, the targeting of IO nanoparticles to cells depends on a number of factors including extracellular IO nanoparticle concentration, particle size, surface coating, and incubation time.

There remain many problems to be addressed in the study of IO nanoparticles for tumor imaging, including 1) the optimal number of targeted ligands on IO nanoparticles must be investigated and determined in each application, since excessive amounts of targeting ligands on the IO nanoparticles may not necessarily increase binding of the IO nanoparticles to specific cells, but can increase the size of the nanoparticles and further affect the R2 characteristics. The ideal ratio of ligands and IO nanoparticles may be dependent on the number of receptors on targeted cells, the binding affinity of ligands to receptors and the molecular weight and size of ligands, 2) the fate of targeted IO nanoparticles after cell internalization is still controversial, with most reports showing that nanoparticles enter into endosomes and are then degraded in lysosomes, while other studies have shown that they can escape from the endosome and locate in the cytoplasm or around the nucleus. It seems that conjugated ligands and surface coating affect the distribution of particles within the cells, 3) the range of the concentration of IO nanoparticles used for animal studies is large, from 1 mg to 250 mg of Fe/kg, making it difficult to compare results from different research groups, 4) the quantification of IO nanoparticle levels \textit{in vivo} is still a challenge. In this case, MRI can be combined with other specific labeling technologies such as radio- and NIR-labeling, which may offer the possibility of multimodal imaging for measuring the biodistribution of targeted IO nanoparticles.

**Strategies to increase sensitivity and specificity of targeted IO nanoparticles for \textit{in vivo} imaging**

Although recent advances have demonstrated the feasibility of using targeted IO nanoparticles for noninvasive imaging in animal models, one of the main problems is that IO nanoparticles are usually taken up by macrophages in the liver (Küpffer cells), spleen and bone marrow, thus affecting...
their specificity and sensitivity and rendering them less than ideal for this application. Previous studies have reported the uptake of dextran-coated monocrystalline iron oxide nanoparticles (MION) ranging from 0.011 to 0.118 pg of iron per cell (1 hr of incubation) by various tumor cells, and a maximum load of 0.97 pg in mouse macrophages (Moore et al 1997). A biodistribution study of MnMEIO-Hereceptin conjugates labeled with radioactive \(^{111}\)In by \(\gamma\)-counter analyses showed that in addition to being distributed in the tumor (3.4% injected dose (ID)/g), nanoparticles were also found in the liver (12.8% ID/g), spleen (8.7% ID/g) and muscle (1.0% ID/g) (Lee et al 2007b).

Macrophages are capable of internalizing a wide variety of materials including iron oxide nanoparticles. There are many different pathways that can regulate the internalization of IO nanoparticles by macrophage cells because of the diversity in particle size, tendency to aggregate and surface coating. Several studies have sought to minimize the non-specific uptake of IO nanoparticles by macrophages (Zhang et al 2002; Rogers and Basu 2005; Leuschner et al 2006; Lee et al 2006).

Rogers and Basu (2005) reported that pretreatment of macrophages with the MG-CoA reductase inhibitor lovastatin (1\(\mu\)M) could significantly reduce SPIO uptake by activated macrophages to 61% of untreated cells. Lovastatin downregulates class A types I and II macrophage scavenger receptors, and may bind to other related receptors in macrophages and reduce receptor recycling, thus partially abolishing IO internalization. The uptake rates of IO nanoparticles by liver and spleen can be decreased by limiting phagocytosis, leading to longer blood half-lives which provide favorable conditions for nanoparticles to reach their targets. Pretreatment with lovastatin before the injection of targeted IO nanoparticles may provide a new method to decrease the nonspecific uptake of targeted IO nanoparticles by the liver or spleen but increase their concentration in the tumor site (Rogers and Basu 2005). Another method to decrease nonspecific uptake of IO nanoparticles is to eliminate plasma opsonins by injecting decoy particles. Simberg and colleagues (2007) found that this treatment caused 5-fold prolongation in particle half-life and that Ni-liposome pretreatment greatly increased tumor homing of the nanoparticles, which primarily localized in tumor blood vessels. However, toxicity limits the further application of this agent.

In general, positively charged nontargeted IO nanoparticles bind to cells through electrostatic interaction with the negatively charged cell membranes and are then internalized by cells, while endocytosis of negatively surface-charged IO nanoparticles may occur through both protein-mediated phagocytosis and diffusion. A change in IO nanoparticle surface charge can be induced by covalently coupling different chemical materials such as amino, PEG and carboxyl groups. It has been reported that albumin-IO nanoparticles with a neutral charge showed a reduced phagocytic uptake in comparison with negatively or positively charged particles (Roser et al 1998). Fang and colleagues (2006) found that the charge of nanoparticles strongly affects both the blood circulation time and the bioavailability of particles within the body. The surface charge of IO nanoparticles should ideally be maintained at neutral or close to neutral for imaging and drug delivery (Shi et al 2007).

In addition, the size of IO nanoparticles will potentially affect their distribution in vivo. Intravenously injected nanoparticles with diameters greater than 200 nm are usually taken up by the liver and spleen, and are eventually removed by the cells of the RES, resulting in decreased blood circulation times (Remsen et al 1996). Smaller particles with diameters less than 5 nm are rapidly removed through the kidney (Gupta and Gupta 2005), therefore, IO nanoparticles ranging from 5 to 150 nm may offer the most effective distribution in certain tissues, especially in tumors.

To develop tumor targeted-IO nanoparticles that have both high sensitivity and specificity remains challenging. Despite many recent advances in the development of targeted IO nanoparticles for tumor imaging, we are still limited in our ability to detect tumors at their early stages of development, to monitor their invasion and metastasis and to assess their responses to therapy.

**Tumor-targeted IO nanoparticles as selective drug delivery vehicles**

Targeted IO nanoparticles can be used to treat tumors in three different ways. Firstly, specific antibodies can be conjugated to the IO nanoparticles to selectively bind to related receptors and inhibit tumor growth (Huh et al 2005). Secondly, targeted IO nanoparticles can be used for hyperthermia for tumor therapy (DeNardo et al 2005; Sonvico et al 2005; Jordan et al 2006). Thirdly, drugs can be loaded onto the IO nanoparticles for targeted therapy. In this review, we focus on selective drug delivery by targeted IO nanoparticles.

Increasing evidence shows that the selective delivery of therapeutic agents into a tumor mass may minimize toxicity to normal tissues and improve bioavailability of cytotoxic agents (Shenoy et al 2005; Gang et al 2007; Bae et al 2007; Lee et al 2007c).
There are several strategies to incorporate drugs into targeted IO nanoparticles. Drugs can be linked to the carrier coating, deposited in the surface layer, or trapped within the IO nanoparticles themselves (Chen et al 2007). They can be released by diffusion, vehicle rupture, dissolution or endocytosis of the formulation (Lanza et al 2004; Atri 2006). The imaging signals produced by IO nanoparticles detected by MRI combined with the amount of specific drug contained per particle can be used to estimate the tissue drug levels. In addition, radio- or organic dye-labeled drugs can be loaded into the IO nanoparticles for more accurate quantification of drug distribution in vivo.

Unfortunately, only a few studies have used targeted IO nanoparticles as drug delivery carriers, especially for in vivo applications. Methotrexate (MTX) is an analogue of FA, which can exhibit both a targeting role as FA and a therapeutic effect in cancer cells that overexpress folate receptor on their surface. Kohler and colleagues (2005) conjugated MTX to IO nanoparticles through amidation between the carboxylic acid end groups on MTX and the amine groups on the particle surface. Their results showed that cells expressing the human folate receptor internalized a higher level of MTX-IO nanoparticles than negative control cells. This MTX-conjugated IO nanoparticle has several advantages, 1) high drug loading efficiency, the average number of MTX molecules per IO nanoparticle with a 10 nm diameter was about 418.9, 2) selective internalization of the targeted IO nanoparticles in tumor cells overexpressing the folate receptor, 3) MTX released only from the IO nanoparticles within lysosomes inside the targeted cells at low pH by cleavage of the amide, 4) drug delivery to the tumor sites may be monitored in vivo by MRI in real-time (Kohler et al 2005).

Polymeric micelles are self-assembled nanoparticles from amphiphilic block copolymers, which have unique characteristics such as high water-solubility, high drug loading capacity and low toxicity. Nasongkla and colleagues (2006) developed novel multifunctional polymeric micelles by loading SPIO nanoparticles inside the micelles at 6.7 w/w %. The chemotherapeutic agent doxorubicin (DOXO) was also loaded at 2.7 w/w % in the micelles and could be released through a pH-dependent mechanism. One of the advantages of the multifunctional nanoparticles is that the encapsulation of DOXO and SPIO nanoparticles inside the hydrophobic micelle cores can avoid potential exposure of hydrophobic SPIO surfaces and adsorption of blood proteins, thus decreasing nonspecific uptake by RES. In addition, the cRGD ligand that can target \( \alpha_v\beta_3 \) integrins on tumor endothelial cells was attached to the micelle surface via a covalent thiol-maleimide linkage. Once internalized by targeted cells, high concentrations of DOXO were released in cell nuclei. This integrated nanomedicine platform may be an ideal contrast agent for targeted tumor therapy and noninvasive imaging in vivo (Nasongkla et al 2006).

Yang and colleagues (2007) developed a new multifunctional hybrid nanosystem by combining magnetic nanocrystals, anticancer drugs and biodegradable amphiphilic block copolymers. In this study, there were about 41.7 wt% (MnFe\(_2\)O\(_4\)) and 40.9 wt% (Fe\(_3\)O\(_4\)) magnetic nanoparticles in the multifunctional magneto-polymeric nanohybrids (MMPNs), and the amount of DOXO in the HER-MMPNs and entrapment efficiency were 3.3 wt% and 71.4%, respectively. In addition, anti-HER antibody was conjugated to the MMPNs by utilizing the carboxyl group on the surface of the particles. As shown in Figure 3, the injected HER-MMPNs were delivered in a target-specific manner to overexpressed HER2/neu receptors on NIH3T3T6.7 cells in vivo and were taken up by a receptor-mediated endocytosis process. The HER-conjugated MMPNs showed significant synergistic effects on inhibition of tumor growth by DOXO. The antibody-conjugated nanoparticles also demonstrated ultrasensitive targeted detection by MRI in both in vitro and in vivo models (Yang et al 2007).

However, there are still many obstacles for successfully using tumor-targeted IO nanoparticles as drug carriers in vivo, 1) functional group modification of the drugs during conjugation may change their chemical properties, 2) lower drug loading efficiency, 3) quick release of conjugated or encapsulated drugs from IO nanoparticles in the blood before entering into tumor mass, 4) drugs usually released in the endosome or lysosome but not in the cytoplasm within targeting cells, 5) embedding part of the ligand binding site in IO nanoparticles may decrease the targeting ability, 6) loss in magnetization of the core magnetic material during multistep chemical reaction (Jain et al 2005). IO nanoparticles combined with other nanoparticles such as biodegradable and biocompatible polymeric micelles may overcome some of the above obstacles. Proper surface coating of IO nanoparticles and methods for the more effective loading of anticancer drugs will facilitate drug release profiles.

**Conclusions**

Although recent advances have demonstrated the feasibility of using targeted magnetic IO nanoparticles for tumor imaging and therapy, methods and strategies to produce tumor-targeted imaging probes with a high specificity and sensitivity are still greatly needed. There are many
Since IO particles have been used in clinical settings for many years, there is a high potential that these targeted probes will be applicable in clinical applications in the future.

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