Published in final edited form as:

J Mater Chem. 2009 ; 19: 6367–6372. doi:10.1039/b902358e.

A Novel Strategy for Surface Modification of Superparamagnetic Iron Oxide Nanoparticles for Lung Cancer Imaging

Gang Huang^a, Chunfu Zhang^{a,b}, Shunzi Li^{a,c}, Chalermchai Khemtong^a, Su-Geun Yang^a, Ruhai Tian^a, John D. Minna^{a,d}, Kathlynn C. Brown^{a,c}, and Jinming Gao^a

Jinming Gao: jinming.gao@utsouthwestern.edu

^a Department of Pharmacology, Harold C. Simmons Comprehensive Cancer Center, UT Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd. Dallas, Texas 75390, USA Fax: +1 214 645 6347; Tel: +1 214 645 6370

^c Department of Internal Medicine, Division of Translational Research, UT Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd. Dallas, Texas 75390, USA Fax: +1 214 645 6347; Tel: +1 214 645 6370

^d Hamon Center for Therapeutic Oncology, UT Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd. Dallas, Texas 75390, USA Fax: +1 214 645 6347; Tel: +1 214 645 6370

Abstract

Superparamagnetic iron oxide (SPIO) nanoparticles are widely used in magnetic resonance imaging (MRI) as versatile ultra-sensitive nanoprobes for cellular and molecular imaging of cancer. In this study, we report a one-step procedure for the surface functionalization of SPIO nanoparticles with a lung cancer-targeting peptide. The hydrophobic surfactants on the as-synthesized SPIO are displaced by the peptide containing a poly(ethylene glycol)-tethered cysteine residue through ligand exchange. The resulting SPIO particles are biocompatible and demonstrate high T_2 relaxivity. The nanoprobes are specific in targeting $\alpha_v\beta_6$ -expressing lung cancer cells as demonstrated by MR imaging and Prussian blue staining. This facile surface chemistry and the functional design of the proposed SPIO system may provide a powerful nanoplatform for the molecular diagnosis of lung cancer.

Introduction

Lung cancer is the most common cause of cancer-related death worldwide, with 1.4 million deaths occurring annually.¹ It is also the leading cause of cancer death in the US, expected to account for 31% and 26% mortality in men and women in 2008, respectively.² Development of advanced imaging probes that provide information at the molecular and cellular levels is crucial for the molecular diagnosis and targeted therapy of lung cancer. Recently, superparamagnetic nanoparticles (e.g., Fe_3O_4 ,^{3–5} MnFe_2O_4 ⁶) have received considerable attention as molecular imaging probes with substantially higher molar relaxivities over small molecular T_1 agents (e.g., Gd-DTPA). Considerable effort has been devoted to strategies to increase the cancer specificity of superparamagnetic probes to target unique molecular markers of solid tumors.^{7–10} Surface functionalization of the superparamagnetic iron oxide (SPIO) nanoparticles has provided multiple examples of targeted contrast agents that specifically recognize tumor markers such as transferrin receptor,¹¹ folate receptor,¹² and Her-2/neu.^{6, 13} Despite these success, the current modification strategies may suffer from several

Correspondence to: Jinming Gao, jinming.gao@utsouthwestern.edu.

^bCurrent address: Med-X Research Institute, Shanghai Jiaotong University, Shanghai, China

limitations. For example, although coating of SPIO nanoparticles with a silica¹⁴ or gold¹⁵ shell can greatly facilitate ligand attachment, the added layer may compromise the efficiency of SPIO in relaxing the surrounding water molecules. On the other hand, most current surface functionalization procedures require several synthetic steps with reactive intermediates, which makes it inconvenient for the wide use of SPIO in cancer molecular imaging applications.

In this study, we report a one-step method to functionalize the surface of SPIO particles with a lung cancer-targeting peptide (LCP, Scheme 1). The peptide, named H2009.1 (RGDLATLRQL), was isolated from a phage-displayed peptide library by biopanning on the lung adenocarcinoma cell line H2009.^{16, 17} This peptide binds to the restrictively expressed integrin, $\alpha_v\beta_6$, which is up-regulated in many human non-small cell lung carcinomas compared with normal lung tissue.¹⁸ In order to incorporate this peptide onto the SPIO surface, the peptide was synthesized with a poly(ethylene glycol) (PEG₁₁) linker followed by a cysteine residue at the C-terminus of the peptide (NH₂-RGDLATLRQL-PEG₁₁-Cys-COOH, or LCP-PEG-Cys). Here we demonstrate the peptide was able to replace the hydrophobic surfactants on the as-synthesized SPIO nanoparticles through direct ligand exchange. The resulting nanoparticles have shown specific targeting to the $\alpha_v\beta_6$ -positive H2009 cells over $\alpha_v\beta_6$ -negative H460 cells as well as over a scrambled peptide (SP, with the sequence of DALRLQGTLR-PEG₁₁-Cys-COOH, or SP-PEG-Cys)-SPIO control. The facile surface chemistry and ability to incorporate the SPIO chelator as part of solid phase peptide synthesis have the potential to greatly facilitate the use of SPIO for molecular imaging applications.

Experimental

Materials

Fmoc-amino polyethylene glycol propionic acid (C₄₂H₆₅NO₁₆, Fmoc-NH-(PEG)₁₁-COOH) was purchased from Polypure, Oslo, Norway. Fmoc-Cys(Trt)-Wang resin (substitution level 0.59 mmol/g) and all other amino acids were obtained from EMD Biosciences Inc. Dimethylformamide (DMF) and trifluoroacetic acid (TFA) were purchased from Applied biosystems Inc. O-Benzotriazole-N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU) and N-Hydroxybenzotriazole (HOBt) were obtained from Novabiochem. All the other reagents were purchased from Sigma-Aldrich.

Synthesis of superparamagnetic iron oxide particles

Oleic acid-coated hydrophobic SPIO nanoparticles were prepared by thermal decomposition of ferric acetylacetonate (Fe(acac)₃) according to a previously published procedure.¹⁹ Briefly, Fe(acac)₃ (1 mmol), oleic acid (3 mmol), oleylamine (3 mmol) and 1,2-hexadecanediol (5 mmol) were dissolved in 10 mL phenyl ether. The mixture was heated under nitrogen environment at 200 °C for 2 hrs before refluxing the solution at 300 °C for another 1 hr. SPIO particles were precipitated with 20 mL ethanol and resuspended in 10 mL hexane. Uniform SPIO particles (6.2±0.9 nm in diameter) were obtained by sequential centrifugations and ethanol precipitations. The size of these hydrophobic SPIO particles were determined by transmission electron microscopy (TEM, JEOL 1200EX II, JOEL USA, Inc., Peabody, MA) and calculated by averaging the size of 300 particles measured by Image J software.

Syntheses of LCP-PEG-Cys and SP-PEG-Cys peptides

Peptide syntheses were carried out on a SymphonyTM Synthesizer (Rainin Instruments, Protein Technologies, Inc. Woburn, MA) by Fmoc solid phase peptide synthesis using Fmoc-Cys(Trt)-Wang resin. Fmoc-protected amino acids were coupled at a 5-fold excess using HBTU, HOBt and N-methylmorpholine (NMM) for coupling (45 min). 20% piperidine in DMF was used to remove N-terminal Fmoc protecting groups. Fmoc-NH-(PEG)₁₁-COOH was coupled in the same fashion. Upon completion of the synthesis, peptides were cleaved from the resin using a

TFA: 1,2-ethanedithiol (EDT): H₂O: triisopropylsilane cocktail (94:2.5:2.5:1) and precipitated in cold diethyl ether. Crude peptides were purified by reverse phase HPLC using an Aapptec Spirit peptide C18 column (250 mm × 21.2 mm, 5 μm). The purified peptides were analyzed by analytical RP-HPLC (Varian, RP-C18 column, 250mm×4.6mm, 5 μm) and were greater than 95% purity. The peptide molecular weights were confirmed by MALDI-MS (Voyager DETM Pro instrument, Applied Biosystems Inc, Carlsbad, CA).

Surface modification of SPIO nanoparticles

In a typical procedure, SPIO nanoparticles (2.0 mg) were dispersed in 200 μL toluene and mixed with LCP/SP-PEG-Cys (2.0 mg) dissolved in dimethylsulfoxide (DMSO) (200 μL). The mixture was kept at 60 °C for 2 hrs under nitrogen environment. After cooling to room temperature, the peptide-coated SPIO was precipitated with a mixture of ethanol, chloroform and hexane (1:1:2) and redispersed in water. The size and morphology of the peptide-coated SPIO particles were characterized with transmission electron microscopy and dynamic light scattering (DLS 802, Viscotek, Houston, TX). Peptide quantities binding on the surface of SPIO particles were analyzed by the W.M. Keck Facility, Yale University. The magnetic properties of peptide and oleic acid coated particles were measured from the hysteresis curves of air-dried SPIO particles by using alternate gradient magnetometer (AGM, MicroMag 2900, Princeton Measurements, NJ). The T₂ relaxation times were measured by a 0.47 T relaxometer (Maran Ultra, UK) with standard Carr-Purcell-Meiboom-Gill (CPMG) sequence and inversion-recovery technique.

Cell uptake and viability studies

Human lung cancer cell lines, α_vβ₆-positive H2009 cells and α_vβ₆-negative H460 cells, were used for all the *in vitro* biological studies.²⁰ The cells were maintained in an RPMI culture medium supplemented with 5% fetal bovine serum and 1% penicillin and streptomycin (37 °C, 5% CO₂). For cell uptake studies, H2009 and H460 cells were plated in 8-well chamber slides (Poly-D-Lysine Cellware CultureSlide, BD Bioscience, Bedford, MA) and incubated with culture medium containing LCP-SPIO or SP-SPIO at Fe concentrations of 0.03 mM for 0.5 and 2 hrs. After incubation, the media was removed and cells were washed with PBS three times. Subsequently, cells were fixed with methanol for 5 min and acetone for 1 min at -20 °C. For staining, the fixed cells were incubated with 10% Prussian blue for 5 mins and then a mixture of 10% Prussian blue and 20% hydrochloride acid (v/v, 1:1) for 30 mins. The cells were counter stained with nuclear fast red for 5 mins. Images were acquired with a Leica fluorescent microscope (Leica DM5500 B, Chicago, IL).

For viability studies, H2009 and H460 cells were incubated at different Fe concentrations (0.03 or 0.3 mM) of LCP-SPIO or SP-SPIO for 24 hrs. The cell viability was evaluated by using a CellTiter-GloTM Luminescent Cell Viability Assay kit (Promega, Madison, WI). Cell viability was calculated by comparing the number of viable cells incubated with SPIO samples over that from the cell medium control. The standard error was obtained from 3 replicate experiments.

MRI evaluation of SPIO uptake in cancer cells

H2009 lung cancer cells were grown in tissue culture flasks and incubated with LCP-SPIO or SP-SPIO nanoparticles at the Fe concentration of 0.03 mM for 0.5 and 2 hrs. After incubation, H2009 cells were washed with PBS three times, harvested by trypsinization and suspended in 100 μL 1% agarose gel. T₂-weighted images and T₂ relaxation times of cell suspensions were acquired on a Varian INOVA 4.7 T scanner. A spin echo sequence (TR = 5000 ms, TE = 45 and 60 ms, FOV = 35×35 mm, matrix = 128×128, slice thickness = 2 mm) was used for image acquisition.

Results

Surface modification and characterizations of SPIO nanoparticles

We chose to use the PEG-tethered Cys strategy to modify the surface of SPIO nanoparticles (Scheme 1). The thiol and carboxylate groups on Cys can form bi-dentate binding to the Fe ions on the SPIO surface.^{21, 22} Furthermore, oxidation of thiol groups can lead to crosslinked disulfide between Cys residues on the SPIO surface,^{23–25} which can further increase the stability of ligand functionalization.

Figure 1A demonstrates the success of SPIO surface modification strategy by the PEG-Cys method. The as-synthesized SPIO nanoparticles were coated with oleic acids at the surface and consequently very hydrophobic.¹⁹ Partition of these SPIO nanoparticles between the hexane and aqueous phases (the top and bottom layers in Fig. 1A, respectively) shows that most of the particles stayed in the hexane phase (a in Fig. 1A). Treatment of as-synthesized SPIO nanoparticles with LCP peptide (NH₂-RGDLATLRQL-COOH) without the PEG-Cys group did not change the partition behavior of the SPIO nanoparticles (b in Fig. 1A). In comparison, surface treatment with PEG-Cys and LCP-PEG-Cys molecules resulted in complete partitioning of SPIO nanoparticles in the aqueous phase (c and d in Fig. 1A). When the -COOH group of Cys was replaced with -CONH₂, the resulting LCP-PEG₁₁-Cys-CONH₂ was not able to bind on the surface of SPIO particles through ligand exchange, therefore failed to solubilize the SPIO particles in water under otherwise identical surface modification conditions (data not shown).

The attachment of PEG-Cys on the SPIO surface was further confirmed by the FTIR. As-synthesized SPIO nanoparticles have shown characteristic Fe-O vibration bands at 600 and 450 cm⁻¹ (Fig. 1B).²⁶ In addition, surface-bound oleic acids can be observed by the presence of 2900 (C-H stretching) and 1620 (C=O stretching) cm⁻¹ peaks. In the PEG-Cys modified SPIO sample, the Fe-O absorption bands were retained. Moreover, absorption peaks due to PEG-Cys (e.g., 1100 and 1660 cm⁻¹ peaks from PEG C-O-C and Cys C=O stretching bands, respectively) were also observed. Based on the amino acid analysis of the peptides on the SPIO particles, we calculated that approximately 100–130 LCP(SP)-PEG-Cys chains were bound per 6 nm SPIO particle.

The magnetic properties of the SPIO nanoparticles did not change significantly upon ligand exchange. Magnetic hysteresis data showed almost superimposable magnetization curves for the as-synthesized and LCP-PEG-Cys modified SPIO nanoparticles (Fig. 1C). The values of saturated magnetization moment (M_s) are approximately 90 emu/g Fe for both samples. This data suggests that ligand exchange on the surface of SPIO did not considerably affect the SPIO nanocrystalline structure and magnetic properties.

The LCP/SP-PEG-Cys modified SPIO nanoparticles were further characterized by the TEM and DLS (Fig. 2). TEM data showed that both SPIO samples were singly dispersed on the copper grids without the formation of clustered SPIO aggregates. The average diameters were 6.2±0.9 and 6.4±1.1 nm for the LCP-SPIO and SP-SPIO, respectively. The DLS histograms (Figs. 2B and 2D) showed a single population of particle size distribution for both samples. The hydrodynamic diameters were 16.2±2.5 and 15.7±3.1 nm for the LCP-SPIO and SP-SPIO, respectively. The larger diameter from the DLS measurement reflects the contribution of the LCP/SP-PEG-Cys surface coatings (they are not visible under TEM) to the increase in hydrodynamic size.

Cell uptake and viability of LCP/SP-SPIO nanoparticles

To determine the targeting specificity of LCP-SPIO nanoparticles to $\alpha_v\beta_6$ -positive lung cancer cells, we performed two experiments. In the first experiment, we compared the cell uptake

efficiency of LCP-SPIO nanoparticles between $\alpha_v\beta_6$ -positive H2009 and $\alpha_v\beta_6$ -negative H460 cells. Prussian blue staining showed considerably increased Fe uptake in $\alpha_v\beta_6$ -positive H2009 cells over $\alpha_v\beta_6$ -negative H460 cells (Fig. 3A). Majority of the particles appear to be localized in the perinuclear region of the cell, consistent with the previous observation with LCP-modified quantum dots in H2009 cells.¹⁸ In the second experiment, we compared the uptake efficiency in H2009 cells between the LCP-SPIO and SP-SPIO nanoparticles. Results show that cell targeting is peptide specific as the peptide with scrambled sequence did not mediate significant SPIO uptake in H2009 cells. These results indicate that surface-bound LCP peptides maintained their biological activities and achieved cancer-specific targeting properties for the SPIO nanoprobe.

In order to evaluate whether peptide functionalization may induce toxicity to the cells, LCP-SPIO or SP-SPIO at two different Fe concentrations were incubated with H2009 or H460 cells for 24 hours. Under these conditions, neither of the particles showed observable cytotoxicity (Fig. 3B).

Evaluation of SPIO targeting specificity to lung cancer cells by MRI

Prior to evaluation of SPIO targeting specificity to lung cancer cells, we examined and compared the MR relaxivities of the SPIO nanoparticles between LCP-SPIO and SP-SPIO. At 37 °C, both particle formulations showed similar MR relaxivities (Fig. 4A). More specifically, the T_1 and T_2 relaxivities were measured to be 11.5 and 142 s⁻¹ Fe mM⁻¹ for LCP-SPIO, respectively. In comparison, the T_1 and T_2 relaxivities were 12.1 and 141 s⁻¹ Fe mM⁻¹ for SP-SPIO, respectively. Thus, the particles show comparable intrinsic MR properties regardless of which peptide is conjugated at the surface.

MR imaging of H2009 cancer cells incubated with LCP-SPIO showed significantly reduced T_2 relaxation times over the SP-SPIO control (Fig. 4B). T_2 relaxation times were 27.3±3.2 and 45.0±5.2 ms after 0.5 hrs incubation for LCP-SPIO and SP-SPIO, respectively ($p = 0.05$). The difference is even more significant after 2 hrs incubation ($p = 0.01$), where T_2 relaxation times were 16.7 ±2.5 and 35.6±3.1 ms for LCP-SPIO and SP-SPIO, respectively. These results are consistent with the observation from the cell uptake studies (Fig. 3A), which demonstrates the $\alpha_v\beta_6$ -targeting specificity of LCP-SPIO nanoprobe.

Discussion

Lung cancer is the leading cause of cancer-related deaths in the US and its five year survival rate is less than 15%.² When lung cancer is diagnosed early, surgical resection, radiation and chemotherapy can substantially improve survival. Currently, computed tomography (CT) and positron emission tomography (PET) are frequently used for lung tumor diagnosis.²⁷ These methods have shown limited accuracy (e.g. PET suffers from low spatial resolution)²⁸ and specificity (e.g. CT has high false positive rates²⁹) for the diagnosis of lung cancer. The current study aims to develop highly specific SPIO nanoprobe and use the high spatial resolution capability of MRI for molecular diagnosis of lung cancer.

SPIO nanoparticles are the most commonly used T_2 contrast agents used in the clinics. Several different formulations (e.g. Feridex IV, Resovist) are clinically used for liver or GI tract imaging.³⁰ Most of these particles were synthesized by the low-temperature hydrolytic procedure that utilized alkaline precipitation of Fe²⁺/Fe³⁺ salts. Their applications in cancer molecular imaging may be limited due to the high polydispersity of SPIO nanoparticles, low relaxation rates and lack of specificity *in vivo*. Recently, much attention has been devoted to the development of structurally well-defined superparamagnetic nanoparticles with high magnetization and MRI sensitivity.³¹ High temperature nanocrystallization methods with Fe(CO)₅ or Fe(acac)₃ as precursors in the presence of surfactants (e.g. oleic acid) were established

to yield SPIO nanoparticles with narrow size distributions.^{19, 32} In addition to Fe₃O₄, other SPIO compositions (e.g. MnFe₂O₄,⁶ Zn_xFe_{3-x}O₄, x<0.5³³) have been synthesized with increased magnetization and T₂ relaxivity.

Our current surface functionalization strategy employs the use of monodisperse SPIO nanoparticles coated with hydrophobic surfactants. The proposed surface chemistry has several potential advantages. First, the procedure can lead to stably dispersed single SPIO particles in aqueous solution as demonstrated by the TEM and DLS. Because a defined PEG length of only 11 ethylene glycol units was used, the hydrodynamic size of the modified SPIO particles was narrowly distributed with only a few nanometer increase compared with inorganic Fe₃O₄ core. The relatively small hydrodynamic diameter (~16 nm) of the resulting SPIO nanoprobe may prove important in targeting lung cancer cells with enhanced tissue penetration properties. Second, after surface modification, the SPIO nanoparticles maintained its magnetic properties as demonstrated by the hysteresis curves (Fig. 1C). The direct conjugation strategy should also maintain the high intrinsic MR relaxivities of SPIO nanoparticles without possible attenuations by the introduction of a non-magnetic layer (e.g. SiO₂ or Au).^{14, 15} Third, the PEG-Cys design has resulted in stable ligand conjugation on the SPIO surface, which helped achieve the α_vβ₆-specific targeting of lung cancer cells. The bidentate chelation^{21, 22} and possible disulfide crosslinking^{23–25} at the SPIO surface provide the thermodynamic driving force for the stable surface attachment of biological molecules. Similarly, Sun and coworkers have also utilized a bidentate strategy with 4-methylcatechol to achieve stable surface functionalization of SPIO particles.⁵ Finally, our PEG-Cys design can be easily incorporated in a solid phase peptide synthesis protocol, which can be generalized to a variety of small peptide molecules. This strategy provides a complementary approach to the use of amphiphilic micelle strategy for the solubilization and ligand modification of SPIO nanoprobe.^{34–36} Further work is in progress to investigate the long-term stability of the surface coating, shelf-life and *in vivo* imaging efficacy of these SPIO nanoprobe.

Conclusions

In summary, we describe a facile chemical method to functionalize the surface of SPIO nanoparticles for molecular imaging of lung cancer. A lung cancer targeting peptide, H2009.1, was successfully introduced at the SPIO surface when a PEG-cysteine moiety was added to the carboxy-terminus of the peptide. The resulting SPIO nanoprobe demonstrated α_vβ₆-specific targeting of human H2009 lung cancer cells over the α_vβ₆-negative H460 control by Prussian blue staining and T₂-weighted MR imaging. The relatively small size, monodisperse size distribution, and versatile surface chemistry open up many opportunities for the implementation of biologically specific SPIO nanoprobe for molecular diagnosis of lung cancer.

Acknowledgments

This research is supported by the NIH grants R21EB005394 and R01CA129011 to JG, R01CA106646 and Welch (I1622) grants to KCB, and Lung Cancer SPORE P50CA70907 to JDM. GH is supported by a Susan G. Komen foundation postdoctoral fellowship (PDF0707216). CK is supported by a DOD Breast Cancer Research Program Multidisciplinary Postdoctoral Award (W81XWH-06-1-0751). SGY is partially supported by a Korean Research Foundation Grant (MOEHRD, KRF-2006-214-E00039). MR imaging was facilitated by the NCI SW-SAIR grant (U24 CA126608). This is CSCN045 from the program in Cell Stress and Cancer Nanomedicine, Simmons Comprehensive Cancer Center, UT Southwestern Medical Center.

Notes and references

1. WHO. Cancer. 2008. www.who.int
2. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. CA Cancer J Clin 2008;58:71–96. [PubMed: 18287387]

3. Jun YW, Lee JH, Cheon J. *Angew Chem Int Ed Engl* 2008;47:5122–5135. [PubMed: 18574805]
4. Laurent S, Forge D, Port M, Roch A, Robic C, Vander Elst L, Muller RN. *Chem Rev* 2008;108:2064–2110. [PubMed: 18543879]
5. Xie J, Chen K, Lee HY, Xu C, Hsu AR, Peng S, Chen X, Sun S. *J Am Chem Soc* 2008;130:7542–7543. [PubMed: 18500805]
6. Lee JH, Huh YM, Jun YW, Seo JW, Jang JT, Song HT, Kim S, Cho EJ, Yoon HG, Suh JS, Cheon J. *Nat Med* 2007;13:95–99. [PubMed: 17187073]
7. Park J, Von Maltzahn G, Zhang L, Schwartz MP, Ruoslahti E, Bhatia SN, Sailor MJ. *Adv Mater* 2008;20:1630–1635.
8. Nasongkla N, Bey E, Ren J, Ai H, Khemtong C, Guthi JS, Chin SF, Sherry AD, Boothman DA, Gao J. *Nano Lett* 2006;6:2427–2430. [PubMed: 17090068]
9. Amirbekian V, Lipinski MJ, Briley-Saebo KC, Amirbekian S, Aguinaldo JG, Weinreb DB, Vucic E, Frias JC, Hyafil F, Mani V, Fisher EA, Fayad ZA. *Proc Natl Acad Sci U S A* 2007;104:961–966. [PubMed: 17215360]
10. Khemtong C, Kessinger CW, Ren J, Bey E, Yang S, Guthi JS, Boothman DA, Sherry AD, Gao J. *Cancer Res* 2009;69:1651–1658. [PubMed: 19190328]
11. Hogemann-Savellano D, Bos E, Blondet C, Sato F, Abe T, Josephson L, Weissleder R, Gaudet J, Sgroi D, Peters PJ, Basilion JP. *Neoplasia* 2003;5:495–506. [PubMed: 14965443]
12. Sonvico F, Mornet S, Vasseur S, Dubernet C, Jaillard D, Degrouard J, Hoebeke J, Duguet E, Colombo P, Couvreur P. *Bioconjug Chem* 2005;16:1181–1188. [PubMed: 16173796]
13. Artemov D, Mori N, Okollie B, Bhujwalla ZM. *Magn Reson Med* 2003;49:403–408. [PubMed: 12594741]
14. Lee J, Lee Y, Youn JK, Na HB, Yu T, Kim H, Lee SM, Koo YM, Kwak JH, Park HG, Chang HN, Hwang M, Park JG, Kim J, Hyeon T. *Small* 2008;4:143–152. [PubMed: 18189246]
15. Mandal M, Kundu S, Ghosh SK, Panigrahi S, Sau TK, Yusuf SM, Pal T. *J Colloid Interface Sci* 2005;286:187–194. [PubMed: 15848416]
16. Guan H, McGuire MJ, Li S, Brown KC. *Bioconjug Chem* 2008;19:1813–1821. [PubMed: 18710273]
17. Oyama T, Sykes KF, Samli KN, Minna JD, Johnston SA, Brown KC. *Cancer Lett* 2003;202:219–230. [PubMed: 14643452]
18. Elayadi AN, Samli KN, Prudkin L, Liu YH, Bian A, Xie XJ, Wistuba, Roth JA, McGuire MJ, Brown KC. *Cancer Res* 2007;67:5889–5895. [PubMed: 17575158]
19. Sun S, Zeng H, Robinson DB, Raoux S, Rice PM, Wang SX, Li G. *J Am Chem Soc* 2004;126:273–279. [PubMed: 14709092]
20. Phelps RM, Johnson BE, Ihde DC, Gazdar AF, Carbone DP, McClintock PR, Linnoila RI, Matthews MJ, Bunn PA Jr, Carney D, Minna JD, Mulshine JL. *J Cell Biochem Suppl* 1996;24:32–91. [PubMed: 8806092]
21. Handa, H.; Abe, M.; Hasegawa, M. US Patent. 20050084539. 2005.
22. Borghi EB, Morando PJ, Blesa MA. *Langmuir* 1991;7:1652–1659.
23. Nishio K, Gokon N, Tsubouchi S, Ikeda M, Narimatsu H, Sakamoto S, Izumi Y, Abe M, Handa H. *Chem Lett* 2006;35:974–975.
24. Amirbahman A, Sigg L, Gunten U. *J Colloid Interface Sci* 1997;194:194–206. [PubMed: 9367598]
25. Fauconnier N, Pons JN, Roger J, Bee A. *J Colloid Interface Sci* 1997;194:427–433. [PubMed: 9398425]
26. Ma M, Zhang Y, Yu W, Shen HY, Zhang HQ, Gu N. *Colloids Surf A: Physicochem Eng Asp* 2003;212:219–226.
27. Oken MM, Marcus PM, Hu P, Beck TM, Hocking W, Kvale PA, Cordes J, Riley TL, Winslow SD, Peace S, Levin DL, Prorok PC, Gohagan JK. *J Natl Cancer Inst* 2005;97:1832–1839. [PubMed: 16368945]
28. Townsend DW. *Ann Acad Med Singapore* 2004;33:133–145. [PubMed: 15098626]
29. Toloza EM, Harpole L, McCrory DC. *Chest* 2003;123:137S–146S. [PubMed: 12527573]
30. Wang YXJ, Hussain SM, Krestin GP. *Eur Radiol* 2001;11:2319–2331. [PubMed: 11702180]

31. Park J, Joo J, Kwon SG, Jang Y, Hyeon T. *Angew Chem Int Ed Engl* 2007;46:4630–4660. [PubMed: 17525914]
32. Hyeon T, Lee SS, Park J, Chung Y, Na HB. *J Am Chem Soc* 2001;123:12798–12801. [PubMed: 11749537]
33. Barcena C, Sra AK, Chaubey GS, Khemtong C, Liu JP, Gao J. *Chem Commun (Camb)* 2008:2224–2226. [PubMed: 18463747]
34. Ai H, Flask C, Weinberg B, Shuai X, Pagel MD, Farrell D, Duerk J, Gao J. *Adv Mater* 2005;17:1949–1952.
35. Yu WW, Chang E, Sayes CM, Drezek R, Colvin VL. *Nanotechnology* 2006;17:4483–4487.
36. Nitin N, LaConte LE, Zurkiya O, Hu X, Bao G. *J Biol Inorg Chem* 2004;9:706–712. [PubMed: 15232722]

Fig 1.

(A) Solubility test of different surface modified SPIO particles dispersed in a water and hexane mixture (a) SPIO particles, (b) LCP treated SPIO particles, (c) PEG-Cys treated SPIO particles and (d) LCP-PEG-Cys treated SPIO particles; (B) FTIR spectra of oleic acid coated SPIO, free PEG-Cys and PEG-Cys coated SPIO; (C) Magnetic hysteresis loops of surface modified 6nm SPIO particles.

Fig 2. TEM (A, C) and DLS (B, D) characterization of LCP/SP-PEG-Cys modified SPIO nanoparticles. (A, B) SPIO nanoparticles modified by LCP-PEG-Cys. (C, D) SPIO nanoparticles modified by SP-PEG-Cys. The scale bars in A and C are 20 nm.

Fig 3.

Evaluation of cell targeting specificity and cytotoxicity of SPIO nanoprobcs. (A) Prussian blue staining of $\alpha_v\beta_6(+)$ -H2009 and $\alpha_v\beta_6(-)$ -H460 cells treated with LCP-SPIO and SP-SPIO at 0.03 mM Fe concentration for 2 hrs; (B) percentage of cell viability after incubation with SPIO samples at Fe concentrations of 0.03 and 0.3 mM for 24 hours.

Fig 4.

Evaluation of cell targeting specificity of SPIO nanoprobe by MRI. (A) T₁ and T₂ relaxation rates as a function of Fe concentrations for LCP-SPIO and SP-SPIO; (B) T₂ relaxation times of H2009 cells treated with LCP-SPIO and SP-SPIO nanoprobe for 0.5 and 2 hrs. The p values were calculated by Student's t-test and showed statistical significance between the LCP-SPIO and SP-SPIO samples.

Scheme 1.

Surface modification of superparamagnetic iron oxide (SPIO) nanoparticles with LCP-PEG-Cys for lung cancer targeting. A scrambled peptide control (SP-PEG-Cys) is included to examine the $\alpha_v\beta_6$ specificity for cell targeting.