Published in final edited form as:

Curr Opin Biomed Eng. 2017 September ; 3: 67–73. doi:10.1016/j.cobme.2017.11.003.

Magnetic resonance molecular imaging for non-invasive precision cancer diagnosis

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Abstract

Magnetic resonance imaging (MRI) is a non-invasive clinical imaging modality for high-resolution imaging of soft tissues. Magnetic resonance molecular imaging (MRMI) has the potential to provide high-resolution delineation of cancer for precision medicine. However, its clinical application is hampered by the low sensitivity of contrast enhanced MRI and the lack of safe and effective targeted MRI contrast agents. Significant progress has recently been made in the design and development of novel clinically translatable targeted MRI contrast agents for MRMI of cancer. The challenges and strategies for designing the safe and effective targeted MRI contrast agents are discussed here. Some of the recent progresses in MRMI are also highlighted. These progresses provide a new paradigm for the design and development of safe and effective MRI contrast agents for clinical translation and pave the way for clinical application of MRMI in precision management of cancer.

Introduction

Precision medicine offers the promise to tailor personalized therapies to improve healthcare of cancer patients. Precision medicine requires accurate cancer detection and characterization of tumor aggressiveness. Molecular imaging provides non-invasive measurement of cancer biomarkers for accurate cancer detection and characterization. Magnetic resonance imaging (MRI) is routinely used for three-dimensional visualization of soft tissues in high-resolution and for cancer detection and diagnosis. Although MRI is advantageous over other clinical imaging modalities, including nuclear medicine and computed tomography (CT), for high-resolution imaging of cancer biomarkers, its potential is limited by its low sensitivity. Nevertheless, the recent decade has witnessed several groundbreaking advancements in MR molecular imaging (MRMI) of cancer. Novel targeted

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Conflict of interest

Dr. Zheng-Rong Lu is one of the founders of Molecular Theranostics LLC and MoTek Pharmaceuticals, Inc. The companies are focused on the commercialization of targeted imaging agents for cancer.

contrast agents with better safety profiles have been designed and developed to overcome previous limitations for effective MR molecular imaging of cancer [1, 2]. Indeed, high spatial resolution of molecular MRI with newer targeted contrast agents can now facilitate early detection of tumors as small as a few hundred cells, a few hundred microns in size, and risk-stratification of aggressive tumors. This article summarizes the concepts, principles, and recent progress of molecular MRI in cancer imaging.

Current imaging technologies in cancer diagnosis

Early and accurate detection of malignant tumors is a vital step in initiating early effective treatment of the disease. Medical imaging is routinely used for non-invasive cancer detection, monitoring disease progression, and timely assessment of therapeutic efficacy to assist physicians in tailoring better therapies to achieve the best possible therapeutic outcome. Imaging modalities, including mammography, ultrasound, X-ray CT, and MRI, which provide anatomical images of the tissues of interest, are used for cancer detection and localization based on morphological changes. High-resolution anatomical imaging with these modalities is also used for assessing disease progression, therapeutic response, and image-guided interventions. Positron emission tomography (PET) and single-photon emission computed tomography (SPECT) constitute the most commonly used clinical molecular imaging modalities. To visualize biomarkers in cancer, PET or SPECT require imaging probes prepared from radioisotopes emitting positrons or gamma-rays. Both these modalities are highly sensitive for quantitative detection and measurement of cancer markers and are commonly used in the clinical management of cancer. However, they suffer from various drawbacks for cancer imaging and cannot suffice for early accurate detection and characterization of malignant tumors for precision medicine. Dual imaging modalities of PET-CT/MRI and SPECT-CT/MRI have been developed to combine the advantages of sensitive and quantitative molecular imaging of PET and SPECT and high-resolution anatomical imaging by CT and MRI to improve the accuracy of cancer diagnosis [3–5].

The principle of MR molecular imaging

MR molecular imaging has the potential to provide high-resolution, sensitive, and quantitative molecular imaging of cancer markers as a single imaging modality. MRI measures the longitudinal (T_1) and transverse (T_2) relaxation rates of protons (mainly water protons) in the body to produce high-resolution images of soft tissues. Contrast agents based on paramagnetic materials are often used to enhance the image contrast between tumors and the surrounding normal tissues [6, 7]. MRI is used for both high-resolution anatomical imaging and functional imaging of cancer. T_1 -weighted MRI contrast enhanced with gadolinium-based contrast agents provides brighter tumor contrast enhancement and is commonly used in cancer imaging. Contrast enhanced MRI is often used in clinical practice for the characterization of primary tumors, assessment of treatment response, and detection of local recurrence in patients. High-resolution molecular MRI can be achieved to improve accurate detection and differential diagnosis if safe and effective contrast agents specific to cancer markers are available for clinical use. The expression levels of cancer markers can be quantitatively determined with T_1 -mapping and the targeted contrast agents.

Effective molecular MRI requires targeted contrast agents to specifically bind to cancer biomarkers to generate detectable signal enhancement by MRI scanners. Currently, the two main classes of MRI contrast agents, including stable paramagnetic Gd(III) chelates [8] and superparamagnetic iron oxide nanoparticles, have been extensively explored for molecular MRI. Gd-based contrast agents (GBCAs) generate bright signal enhancement in T₁-weighted imaging, and are most commonly used in clinical practice. Superparamagnetic iron oxide nanoparticles are mainly T₂-weighted contrast agents and provide dark enhancement. Targeted MRI contrast agents have been designed and prepared by incorporating targeting moieties, which bind to cancer biomarkers, into Gd(III) based MRI contrast agents or superparamagnetic iron oxide nanoparticles. Unlike nuclear medicine, MRI cannot directly visualize the contrast agents bound to their targets. Binding of the targeted agents increases the relaxation rates of the water protons around the molecular targets to generate signal enhancement for molecular MRI.

Signal enhancement in molecular imaging is non-linearly correlated with the increase in the T₁ and T₂ relaxation rates of water protons around the targeted contrast agents. The increase in the relaxation rates is proportional to the relaxivity and concentration of the contrast agents. Effective molecular MRI requires the binding of a sufficient amount of targeted contrast agents with high relaxivities to generate detectable signal enhancement. Conventional MRI contrast agents have relatively low relaxivities. Targeted nanoparticles with high payload of paramagnetic materials, e.g. Gd(III) chelates and iron oxide, are often used to increase local concentration for detecting molecular targets expressed on the cell surface [9, 10]. The effectiveness of the targeted paramagnetic nanoparticles for MR molecular imaging of cancer has been extensively demonstrated with animal models. However, clinical development of these nanosized targeted contrast agents has been impeded by slow excretion of the targeted contrast agents and consequent safety concerns.

The design and development of safe and effective targeted contrast agents specific to cancer biomarkers are critical for clinical application of molecular MRI. Any targeted contrast agent for clinical translation has to satisfy the safety requirements set forth by the regulatory agencies. Rapid and complete excretion from the body after diagnostic imaging is the ultimate priority in the design of targeted MRI contrast agents. Two approaches can be applied to address the challenges in designing clinically translatable targeted contrast agents for cancer molecular MRI, i.e., to target abundant cancer biomarkers and/or to apply paramagnetic materials with high relaxivities that meet the safety requirements, Figure 1. Molecular targets of high concentration allow the binding of a sufficient amount of small molecular targeted contrast agents based on the existing clinical agents into tumors to generate detectable MR signal enhancement [11]. Identification of suitable molecular targets for molecular MRI requires clear understanding of cancer biology of the targets [12]. Ideally, the markers should have abundant expression only in tumor tissues, and not in the normal tissues. The use of paramagnetic materials with high relaxivities could significantly reduce the dose of the contrast agents to improve the sensitivity of MR molecular imaging [13, 14]. Significant increase in the relaxivities of targeted MRI contrast agents can substantially reduce their doses, which could allow effective MR molecular imaging of the cancer markers of relatively low concentration.

Recently, significant progress has been made in the design of targeted MRI contrast agents using the aforementioned approaches. The feasibility of molecular MRI of cancer using the novel targeted contrast agents has been demonstrated in animal tumor models. The following sections summarize the recent progress in molecular MRI of cancer.

MR molecular imaging of abundant oncoproteins using small molecular targeted contrast agents

Effective MR molecular imaging with a small molecular targeted MRI contrast agent was first achieved by targeting the fibrin-fibronectin (FN) clots in tumor extracellular matrix (ECM) in various mouse tumor models [11, 15]. The targeted contrast agents were synthesized by conjugating Gd(III) based clinical contrast agents to small peptides CLT1 and CREKA, which are specific to the fibrin-FN clots in tumor ECM [16, 17]. Small peptides are advantageous for designing targeted MRI contrast agents because of their easy synthesis, ready access and binding to the molecular targets via rapid diffusion in solid tumors. The small size of the targeted agents facilitates rapid excretion of the agents after diagnostic imaging. The abundance of the markers in the tumor ECM allows easy access and binding of sufficient targeted contrast agents to produce robust signal enhancement in the tumor tissue for molecular MRI. High resolution MRI with the CREKA targeted small molecular contrast agent, CREKA-Tris(Gd-DOTA)₃, enables the detection of breast cancer micrometastases as small as 300 μm in mice bearing metastatic 4T1 breast tumors, exceeding the detection limit of the current clinical imaging modalities [18], Figure 2.

In addition, molecular MRI of abundant oncoproteins associated with tumor aggressiveness shows great promise for accurate detection and risk-stratification of malignant tumors. Extradomain B-fibronectin (EDB-FN) is a cancer-related fibronectin isoform highly expressed in the ECM of various aggressive human cancers, but not in normal tissues [19, 20]. It is a marker of epithelial-to-mesenchymal transition (EMT) and plays a key role in EMT induction, cancer cell stemness, cancer proliferation, and angiogenesis. Moreover, its expression is inversely correlated with patient survival [21, 22]. Molecular MRI of EDB-FN with ZD2-[Gd(HP-DO3A)] can effectively detect and differentiate aggressive prostate tumors in mouse xenograft models [20]. ZD2-[Gd(HP-DO3A)], a small molecular targeted contrast agent specific to EDB-FN, is synthesized by conjugating ZD2 peptide to a clinical contrast agent Gd(HP-DO3A). Figure 3 shows T₁-weighted 2D axial spin-echo MR images and contrast-to-noise ratios (CNR) of PC3 and LNCaP human prostate tumors contrast enhanced with ZD2-[Gd(HP-DO3A)] and a clinical control agent Gd(HP-DO3A) (ProHance) at a dose of 0.1 mmol/kg. ZD2-[Gd(HP-DO3A)] produces strong and prolonged enhancement and high CNR in the metastatic PC3 prostate tumors with high EDB-FN expression, but significantly low tumor enhancement and CNR in the non-metastatic LNCaP prostate tumors. On the other hand, the clinical control agent Gd(HP-DO3A) shows low tumor enhancement and CNR in both the tumor models. The expression levels of EDB-FN can also be quantitatively determined by T₁-mapping in different tumors. The study also shows that the unbound agent undergoes rapid clearance from the body with minimal tissue accumulation of the clinical agent, which is a critical safety parameter for clinical translation. This work is the first to demonstrate the potential of molecular MRI with a small

molecular targeted MRI contrast agent for effective detection and risk-stratification of malignant cancers.

MR molecular imaging of cancer using targeted contrast agents of high relaxivities

Significant efforts have been undertaken to develop paramagnetic materials with high relaxivities to improve the sensitivity of contrast enhanced MRI. Some of the Gd(III) protein complexes possess high T_1 relaxivities in the range of 25 - 50 $\text{mM}^{-1}\text{s}^{-1}$ per Gd at 1.4T [23–25]. Targeted contrast agents based on the Gd(III) protein complexes are able to generate significant signal enhancement for tumor detection with a dose as low as 25 $\mu\text{mol/kg}$. These novel Gd(III) protein complexes also exhibit high stability and minimal tissue accumulation. Comprehensive safety assessment is needed to meet the requirements by the regulatory agencies for clinical translation of the protein-based targeted contrast agents for molecular MRI.

Endohedral gadofullerenes are a class of novel paramagnetic materials with superior relaxivities suitable for the design of targeted MRI contrast agents [13, 26, 27]. Gadofullerenes are generally lipophilic and need to be converted to hydroxylated gadofullerenes to improve their water-solubility and T_1 relaxivities. Magnetization of multiple hydroxyl protons on the surface of hydroxylated gadofullerenes by the encaged Gd(III) ions and the exchange of magnetized protons with bulky water protons result in superior relaxivities, which are at least 20 times those of conventional contrast agents, at the magnetic field strengths of most clinical scanners (1.5 and 3.0T). Hydroxylated gadofullerenes are highly promising for the design of targeted MRI contrast agents of superior relaxivities for sensitive molecular MRI of cancer.

Several hydroxylated gadofullerenes, including Gd@C_{60} , Gd@C_{82} , and $\text{Gd}_3\text{N@C}_{80}$, have been prepared and tested as MRI contrast agents [27–29]. These compounds are mostly T_1 -weighted MRI contrast agents. The r_1 relaxivity of the agents largely depends on the structure and chemical modifications of the gadofullerenes, ranging from 38.5 $\text{mM}^{-1}\text{s}^{-1}$ of $\text{Gd@C}_{60}(\text{OH})_x$ to 81 $\text{mM}^{-1}\text{s}^{-1}$ of $\text{Gd@C}_{82}(\text{OH})_{40}$. Targeted gadofullerenes were prepared by conjugating interleukin-13 to hydroxylated $\text{Gd}_3\text{N@C}_{80}$ for cancer molecular MRI [26]. The targeted agent was shown to preferentially accumulate in brain tumors in mice, resulting in strong tumor enhancement after intravenous injection at a low dose. A similar interleukin-13 targeted hydroxylated $\text{Gd}_3\text{N@C}_{80}$ also shows great promise for detecting chronic post-traumatic osteomyelitis in mice [30].

More recently, ZD2 targeted hydroxylated $\text{Gd}_3\text{N@C}_{80}$, ZD2- $\text{Gd}_3\text{N@C}_{80}$, was synthesized by conjugating the peptide to the metallofullerene as a targeted MRI contrast agent with superior relaxivity for molecular MRI of EDB-FN [31]. ZD2- $\text{Gd}_3\text{N@C}_{80}$ has high r_1 and r_2 relaxivities of 223.8 $\text{mM}^{-1}\text{s}^{-1}$ and 344.7 $\text{mM}^{-1}\text{s}^{-1}$ or 74.6 $\text{mM}^{-1}\text{s}^{-1}$ and 114.9 $\text{mM}^{-1}\text{s}^{-1}$ per Gd(III) ion at 1.5T, respectively. The r_1 relaxivity is almost 20 times that of the clinical agents. The effectiveness of ZD2- $\text{Gd}_3\text{N@C}_{80}$ in the detection and differential diagnosis of aggressive breast cancer was tested in mice bearing metastatic MDA-MB-231 triple negative breast cancer and non-metastatic ER-positive MCF-7 tumors. Figure 4 shows T_1 -weighted

MR images of MDA-MB-231 and MCF-7 tumors before and after injecting ZD2-Gd₃N@C₈₀ at a very low dose of 1.67 μ mol/kg or 5 μ mol-Gd/kg, which is 20 times less than that of the clinical GBAs. Significant tumor enhancement was observed in the MDA-MB-231 tumors, while little enhancement was observed in the MCF-7 tumors or in both tumors injected with non-targeted hydroxylated Gd₃N@C₈₀. ZD2-Gd₃N@C₈₀ greatly improves the sensitivity of molecular MRI for cancer imaging and for accurate detection and differential diagnosis of malignant tumors with high expression of EDB-FN.

The targeted gadofullerenes have the potential to alleviate the toxicity associated with conventional Gd(III) chelate based contrast agents due to release of toxic Gd(III) ions. In the gadofullerenes, Gd(III) ions are enclosed inside the fullerene cages and no free Gd(III) ions can be leaked out. It has been shown that the metallofullerene cages can stably withstand exposure to β -radiation [32,33]. The entrapment of the Gd(III) ions in the fullerene cage prevents their systemic release and direct tissue interaction, which is critical for reducing the toxicity of Gd(III) based contrast agents for clinical translation. Hydroxylated gadofullerenes are around 1.0 nm in size, and smaller than the renal filtration threshold, allowing complete excretion of the materials from the body. In addition, due to their superior, the dose of hydroxylated gadofullerenes can be substantially reduced to further minimize dose dependent toxic side effects. These unique properties enable the targeted gadofullerenes to circumvent the toxicity associated with the conventional Gd-based MRI contrast agents. Taken together, these advantageous features of hydroxylated gadofullerenes overcome the drawbacks of conventional Gd(III) based contrast agents, including low relaxivities and sensitivity and potential toxic side effects related to the release of Gd(III) ions. Targeted hydroxylated gadofullerenes are the next generation of MRI contrast agents for effective molecular MRI in clinical practice.

Conclusions

Significant progress has been made in the MR molecular imaging of cancer. Novel targeted MRI contrast agents have been designed and developed to improve the sensitivity of molecular MRI by targeting abundant cancer markers and using new paramagnetic materials of superior relaxivities. By targeting abundant cancer biomarkers associated with tumor aggressiveness, e.g. EDB-FN, MR molecular imaging will undoubtedly improve the accuracy of early detection and risk-stratification of aggressive cancers. Indeed, some of the small molecular peptide targeted Gd(III) based contrast agents are currently in various stages of clinical translation. The clinical implementation of molecular MRI will provide sensitive and quantitative molecular imaging of cancer markers with high anatomical resolution using a single imaging modality. This will, in turn, minimize the use of multiple diagnostic procedures and imaging modalities that are currently used to improve the accuracy of cancer detection and diagnosis, thereby significantly reducing the cost of diagnostic imaging.

Acknowledgments

I thank Dr. Amita M. Vaidya for proofreading the manuscript.

References

1. Zhou Z, Wu X, Kresak A, Griswold M, Lu ZR. Peptide targeted tripod macrocyclic Gd(III) chelates for cancer molecular MRI. *Biomaterials*. 2013; 34(31):7683–93. [PubMed: 23863450]
- **2. Han Z, Li Y, Roelle S, Zhou Z, Liu Y, Sabatelle R, DeSanto A, Yu X, Zhu H, Magi-Galluzzi C, Lu ZR. Targeted Contrast Agent Specific to an Oncoprotein in Tumor Microenvironment with the Potential for Detection and Risk Stratification of Prostate Cancer with MRI. *Bioconjug Chem*. 2017; 28(4):1031–1040. [PubMed: 28201871]
3. Robertson IJ, Hand F, Kell MR. FDG-PET/CT in the staging of local/regional metastases in breast cancer. *Breast*. 2011; 20(6):491–4. [PubMed: 21807517]
4. Hartenbach M, Hartenbach S, Bechtloff W, Danz B, Kraft K, Klemenz B, Sparwasser C, Hacker M. Combined PET/MRI improves diagnostic accuracy in patients with prostate cancer: a prospective diagnostic trial. *Clin Cancer Res*. 2014; 20(12):3244–53. [PubMed: 24763613]
5. Afshar-Oromieh A, Haberkorn U, Hadaschik B, Habl G, Eder M, Eisenhut M, Schlemmer HP, Roethke MC. PET/MRI with a (68)Ga-PSMA ligand for the detection of prostate cancer. *Eur J Nucl Med Mol Imaging*. 40(10):1629–30.
6. Zhou Z, Lu ZR. Gadolinium-based contrast agents for magnetic resonance cancer imaging, Wiley interdisciplinary reviews. *Nanomedicine and nanobiotechnology*. 2013; 5(1):1–18. [PubMed: 23047730]
7. Bernd H, De Kerviler E, Gaillard S, Bonnemain B. Safety and tolerability of ultrasmall superparamagnetic iron oxide contrast agent: comprehensive analysis of a clinical development program. *Invest Radiol*. 2009; 44(6):336–42. [PubMed: 19661843]
8. Caravan P, Ellison JJ, McMurry TJ, Laufer RB. Gadolinium(III) Chelates as MRI Contrast Agents: Structure, Dynamics, and Applications. *Chem Rev*. 1999; 99(9):2293–352. [PubMed: 11749483]
9. Curtet C, Maton F, Havet T, Slinkin M, Mishra A, Chatal JF, Muller RN. Polylysine-Gd-DTPAn and polylysine-Gd-DOTAn coupled to anti-CEA F(ab')₂ fragments as potential immunocontrast agents. Relaxometry, biodistribution, and magnetic resonance imaging in nude mice grafted with human colorectal carcinoma. *Invest Radiol*. 1998; 33(10):752–61. [PubMed: 9788138]
10. Artemov D, Mori N, Okollie B, Bhujwalla ZM. MR molecular imaging of the Her-2/neu receptor in breast cancer cells using targeted iron oxide nanoparticles. *Magn Reson Med*. 2003; 49(3):403–8. [PubMed: 12594741]
11. Ye F, Wu X, Jeong EK, Jia Z, Yang T, Parker D, Lu ZR. A peptide targeted contrast agent specific to fibrin-fibronectin complexes for cancer molecular imaging with MRI. *Bioconjug Chem*. 2008; 19(12):2300–3. [PubMed: 19053180]
- *12. Zhou Z, Lu ZR. Molecular imaging of the tumor microenvironment. *Adv Drug Deliv Rev*. 2016
13. Bolskar RD. Gadofullerene MRI contrast agents. *Nanomedicine (Lond)*. 2008; 3(2):201–13. [PubMed: 18373426]
14. Shu C, Corwin FD, Zhang J, Chen Z, Reid JE, Sun M, Xu W, Sim JH, Wang C, Fatouros PP, Esker AR, Gibson HW, Dorn HC. Facile preparation of a new gadofullerene-based magnetic resonance imaging contrast agent with high 1H relaxivity. *Bioconjug Chem*. 2009; 20(6):1186–93. [PubMed: 19445504]
15. Ye F, Jeong KE, Parker D, Lu ZR. Evaluation of CLT1-(Gd-DTPA) for MR molecular imaging in a mouse breast cancer model. *Chin J Magn Reson Imaging*. 2011; 2(5):376–381.
16. Wu X, Yu G, Lindner D, Brady-Kalnay SM, Zhang Q, Lu ZR. Peptide targeted high-resolution molecular imaging of prostate cancer with MRI. *American journal of nuclear medicine and molecular imaging*. 2014; 4(6):525–36. [PubMed: 25250202]
17. Tan M, Wu X, Jeong EK, Chen Q, Lu ZR. Peptide-targeted Nanoglobular Gd-DOTA monoamide conjugates for magnetic resonance cancer molecular imaging. *Biomacromolecules*. 11(3):754–61.
- **18. Zhou Z, Qutaish M, Han Z, Schur RM, Liu Y, Wilson DL, Lu ZR. MRI detection of breast cancer micrometastases with a fibronectin-targeting contrast agent. *Nature communications*. 2015; 6:7984.
- *19. Han Z, Zhou Z, Shi X, Wang J, Wu X, Sun D, Chen Y, Zhu H, Magi-Galluzzi C, Lu ZR. EDB Fibronectin Specific Peptide for Prostate Cancer Targeting. *Bioconjug Chem*. 2015; 26(5):830–8. [PubMed: 25848940]

- *20. Han Z, Lu ZR. Targeting fibronectin for cancer imaging and therapy. *Journal of Materials Chemistry B*. 2017; 5(4):639–654. [PubMed: 29270293]
21. Freire-de-Lima L, Gelfenbeyn K, Ding Y, Mandel U, Clausen H, Handa K, Hakomori SI. Involvement of O-glycosylation defining oncofetal fibronectin in epithelial-mesenchymal transition process. *Proc Natl Acad Sci U S A*. 108(43):17690–5.
22. Inufusa H, Nakamura M, Adachi T, Nakatani Y, Shindo K, Yasutomi M, Matsuura H. Localization of oncofetal and normal fibronectin in colorectal cancer. Correlation with histologic grade, liver metastasis, and prognosis. *Cancer*. 1995; 75(12):2802–8. [PubMed: 7773930]
23. Pu F, Qiao J, Xue S, Yang H, Patel A, Wei L, Hekmatyar K, Salarian M, Grossniklaus HE, Liu ZR, Yang JJ. GRPR-targeted Protein Contrast Agents for Molecular Imaging of Receptor Expression in Cancers by MRI. *Scientific reports*. 2015; 5:16214. [PubMed: 26577829]
24. Pu F, Salarian M, Xue S, Qiao J, Feng J, Tan S, Patel A, Li X, Mamouni K, Hekmatyar K, Zou J, Wu D, Yang JJ. Prostate-specific membrane antigen targeted protein contrast agents for molecular imaging of prostate cancer by MRI. *Nanoscale*. 2016; 8(25):12668–82. [PubMed: 26961235]
- *25. Xue S, Yang H, Qiao J, Pu F, Jiang J, Hubbard K, Hekmatyar K, Langley J, Salarian M, Long RC, Bryant RG, Hu XP, Grossniklaus HE, Liu ZR, Yang JJ. Protein MRI contrast agent with unprecedented metal selectivity and sensitivity for liver cancer imaging. *Proc Natl Acad Sci U S A*. 2015; 112(21):6607–12. [PubMed: 25971726]
26. Fillmore HL, Shultz MD, Henderson SC, Cooper P, Broaddus WC, Chen ZJ, Shu CY, Zhang J, Ge J, Dorn HC, Corwin F, Hirsch JI, Wilson J, Fatouros PP. Conjugation of functionalized gadolinium metallofullerenes with IL-13 peptides for targeting and imaging glial tumors. *Nanomedicine (Lond)*. 2011; 6(3):449–58. [PubMed: 21542684]
- **27. Li T, Murphy S, Kiselev B, Bakshi KS, Zhang J, Eltahir A, Zhang Y, Chen Y, Zhu J, Davis RM, Madsen LA, Morris JR, Karolyi DR, LaConte SM, Sheng Z, Dorn HC. A New Interleukin-13 Amino-Coated Gadolinium Metallofullerene Nanoparticle for Targeted MRI Detection of Glioblastoma Tumor Cells. *J Am Chem Soc*. 2015; 137(24):7881–8. [PubMed: 26022213]
28. Toth E, Bolskar RD, Borel A, Gonzalez G, Helm L, Merbach AE, Sitharaman B, Wilson LJ. Water-soluble gadofullerenes: toward high-relaxivity, pH-responsive MRI contrast agents. *J Am Chem Soc*. 2005; 127(2):799–805. [PubMed: 15643906]
29. Zhang J, Ye Y, Chen Y, Pregot C, Li T, Balasubramaniam S, Hobart DB, Zhang Y, Wi S, Davis RM, Madsen LA, Morris JR, LaConte SM, Yee GT, Dorn HC. Gd₃N@C₈₄(OH)_x: a new egg-shaped metallofullerene magnetic resonance imaging contrast agent. *J Am Chem Soc*. 2014; 136(6):2630–6. [PubMed: 24432974]
- *30. Xiao L, Li T, Ding M, Yang J, Rodriguez-Corrales J, LaConte SM, Nacey N, Weiss DB, Jin L, Dorn HC, Li X. Detecting Chronic Post-Traumatic Osteomyelitis of Mouse Tibia via an IL-13R α 2 Targeted Metallofullerene Magnetic Resonance Imaging Probe. *Bioconjug Chem*. 2017; 28(2):649–658. [PubMed: 28061526]
- **31. Han Z WX, Roelle S, Chen C, Schiemann WP, Lu Z-R. Targeted gadofullerene with superior relaxivity for sensitive magnetic resonance molecular imaging and risk-stratification of breast cancer. *Nature Comm*. 2017; 8(1):692.
32. Shultz MD, et al. Encapsulation of a radiolabeled cluster inside a fullerene cage, (177)Lu(x)Lu((3-x))N@C(80): an interleukin-13-conjugated radiolabeled metallofullerene platform. *J Am Chem Soc*. 2010; 132:4980–4981. [PubMed: 20307100]
33. Diener MD, Alford JM, Kennel SJ, Mirzadeh S. (212)Pb@C(60) and its water-soluble derivatives: synthesis, stability, and suitability for radioimmunotherapy. *J Am Chem Soc*. 2007; 129:5131–5138. [PubMed: 17394315]

Highlights

- Effective cancer MRMI can be achieved by targeting abundant markers in tumor microenvironment.
- Small peptide targeted Gd(III) chelates are effective for MRMI of abundant cancer markers.
- Targeted gadofullerenes have superior relaxivities for safe and sensitive MRMI of cancer.
- High-resolution MRMI has the potential to detect microscopic malignant tumors.
- MRMI has the potential to provide non-invasive risk-stratification of aggressive tumors.

Figure 1.
Design of clinically feasible targeted MRI contrast agents for targeting abundant cancer markers to increase local contrast concentration (B) and/or using safe paramagnetic materials of high relaxivity (C).

Molecular Imaging of Micrometastases

Figure 2.

A peptide targeted contrast agent CREKA-Tris(Gd-DOTA)₃ binds to FN-fibrin complexes and produces strong tumor enhancement as compared to the pre-contrast image (left). Co-registration of fluorescence cryo-images (bright field, Cy5, and GFP) with contrast enhanced MR images validates the ability of the agent in detecting microscopic metastatic tumors in MRI (right). Arrows point to the tumor location.

Figure 3. T₁-weighted 2D axial spin-echo MR images (7.0 T) and CNR, in PC3 (A) and LNCaP (B) human prostate tumor xenografts in mice, contrast enhanced by ZD2-[Gd(HP-DO3A)] and the clinical agent Gd(HP-DO3A) (ProHance) at 0.1 mmol-Gd/kg. White arrows point to the tumors and green arrows point to the bladder.

Figure 4.

T₁-weighted 2D axial MR images (1.0 T) of MDA-MB-231 and MCF-7 breast tumors in mice, before and after injection of ZD2-Gd₃N@C80, hydroxylated Gd₃N@C80 at 5 μ mol Gd/kg. White arrowhead points to the tumor.