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MR-Eyes for Cancer: Looking within an Impenetrable Disease

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Abstract

Probe development is a critical component in cancer imaging, and novel probes are making major inroads in several aspects of cancer detection and image-guided treatments. Intrinsic MR probes such as signals from metabolites and their chemical shifts have been used for more than a decade to understand cancer physiology and metabolism. Through the integration of technology, molecular biology, and chemistry, the last few years have witnessed an explosion of extrinsic probes for molecular and functional imaging of cancer that, together with techniques such as CEST and hyperpolarization, have significantly expanded the repertoire of MR techniques in basic and translational investigations of many different aspects of cancer. Furthermore, incorporation of MR probes into multifunctional nanoparticles and multimodality imaging platforms have opened new opportunities for MR in image-guided diagnosis and therapy of cancer. Here we have provided an overview of recent innovations that have occurred in the development of MRI probes for molecular and functional imaging of cancer. Although most of these novel probes are not clinically available, they offer significant promise for future translational applications. In this review, we have highlighted the areas of future development that are likely to have a profound impact on cancer detection and treatment.

Keywords

MR probes; cancer; tumor microenvironment; receptor imaging; theranostics

INTRODUCTION

Cancer continues to confound our best-laid plans and even in the twenty-first century we are trying to understand the complexities of the multiple diseases that are classified as ‘cancer’. While there have been some major successes, even these are presented as probability of survival or increase in survival times, and increases of even a few months are viewed as small triumphs. The impenetrability of cancer arises from the plasticity of cancer cells to adapt to treatments and to co-opt stromal cells and the microenvironment in survival strategies that result in local recurrence or distant metastasis, and frequently in the depletion of normal tissue resources that result in cachexia or wasting away of the body. To effectively treat a disease it is important to understand the disease itself and the

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ramifications of the treatments used. The molecular, metabolic and physiological heterogeneity of tumors is a further confounding factor in cancer detection, prognosis, and delivering effective treatments.

Noninvasive imaging provides the advantage of visualizing the entire tumor, unlike pathological evaluation where, because of the invasiveness of the procedure, biopsy specimens are only taken from 1–2 mm regions. However, to date, other than optical endoscopy, none of the noninvasive imaging modalities has attained the precision of histological microscopic analysis of tissue specimens for molecular characterization. Within the context of other noninvasive imaging modalities, magnetic resonance imaging (MRI) provides exquisite spatial anatomic information because of the abundance of the water signal. In addition, although a number of imaging techniques can be used to study cancer and other diseases, only a handful such as computed tomography (CT), positron emission tomography (PET), single photon emission computed tomography (SPECT), ultrasound, and MRI/magnetic resonance spectroscopy (MRS) are noninvasive and can transition seamlessly from bench-top to bedside. Of these, MRI in combination with MRS and magnetic resonance spectroscopic imaging (MRSI) is significantly expanding our understanding of cancer with an array of methods to investigate cancer at the molecular, functional and holistic levels. At the heart of these capabilities and advances are the development and availability of novel imaging probes that are providing the capabilities of looking within cancers and their microenvironments, and for identifying cancer-specific targeting strategies. MRI and MRS are also being developed for image-guided therapies that target cancer cells but do not inflict damage on normal tissue. Applications developed at the bench-top can be translated into achieving tangible benefits for cancer patients.

A solid tumor consists of cancer cells and a supporting network of stromal cells, immune cells, and extracellular matrix (ECM), loosely termed the tumor microenvironment (TME), that provide the infrastructure for the cancer cells to survive and escape to distant sites through the vasculature and the lymphatics. Chaotic and abnormal vascular and lymphatic networks impose additional physiological and metabolic abnormalities such as hypoxia, acidic extracellular pH (pHe), increased interstitial pressure, and increased glycolysis on already existing aberrancies in metabolism such as altered choline metabolism and aerobic glycolysis. These abnormalities in physiology and metabolism have been studied for more than a decade by the use of ‘intrinsic probes’ that detect signals from metabolites and their environment from chemical shifts in MR spectra, and by ‘extrinsic probes’ for dynamic contrast enhanced (DCE) visualization and characterization of the vasculature.

Recent advances in the development of exogenous probes that are targeted to cancer-specific receptors, physiological environments, the ECM or stromal cells, as well as endogenous probes, are providing new MRI/S capabilities for imaging molecular and cellular targets to improve the accuracy of tumor detection and prognosis. MR probes that detect changes in the intra and extracellular space to characterize oncogenic tissue, receptor expression on cancer cells, and endothelial, and stromal cells within the tumor, protease activity, physiological properties such as pH, oxygen tension, angiogenesis, vascularization can be imaged in preclinical or clinical settings (Figure 1). These probes can also be used to improve metastases detection, and for theranostic imaging (1, 2). Because of the high sensitivity of detection of nuclear imaging, imaging receptor expression has been the traditional domain of nuclear imaging probes. However, despite sensitivity limitations, the availability of MRI probes (3) provides the attractive ability of being used in combination with the tremendous physiological and metabolic imaging strengths of MR techniques to understand the functional effects of receptor expression. In addition, signal amplification strategies and the use of probes, such as ^{19}F probes, that provide excellent target-to-background ratio are being actively developed. However, concern for nephrotoxicity

associated with gadolinium-based contrast agent is also an issue in patients with advanced kidney disease (4). The present review highlights recent significant developments in MR probes related to tumor detection, characterization and treatment, and their applications in cancer diagnosis and therapy.

PROBES FOR CANCER AND STROMAL CELLS

Receptor Expression

Receptors, antigens or proteins expressed on cancer or stromal cell surfaces provide accessible targets for detection as well as for image-guided targeting. Labeled antibodies have been used to image antigens overexpressed by cancer cells, such as Her2/neu in breast cancer (5, 6). MR contrast can be generated by injecting a biotinylated antibody that binds to a specific receptor, followed by administration of avidin linked to gadolinium-diethylenetriaminepentaacetate (Gd-DTPA) that binds to the biotin on the antibody (6). Antibody accumulation can be visualized on T₁-weighted images.

MR probes have also been designed to target the estrogen receptor (ER), overexpressed on breast and ovarian tumors (3, 7, 8). The [estradiol-17 α -ylethynyl(pyridine- 2,6 diyl) bis(methylenenitrilo)] tetrakis(acetate)-gadolinium (EPTA-Gd) probe was used in a mouse model of human breast cancer. Contrast generated by the probe was monitored in ER-transfected (ER-positive) and in wild-type (ER-negative) MDA-MB-231 human breast cancer tumors, implanted orthotopically and showed a specific uptake *in vivo* in ER-positive tumors, compared to the ER-negative tumors.

A recently described contrast agent consisting of superparamagnetic iron oxide (SPIO) nanoparticles for MRI, labeled with Cy5.5 dye for near infrared (NIR) fluorescence imaging, and conjugated to peptides (EPPT) was developed to target the underglycosylated MUC-1 (uMUC-1) tumor antigen that is expressed by more than 90% of breast cancers, and is predictive of chemotherapeutic response (9). This contrast agent termed MN-EPPT was used to assess the effect of doxorubicin in orthotopic human breast cancer xenografts *in vivo*. Tumor-specific accumulation of MN-EPPT allowed the assessment of the reduction in tumor volume induced by the treatment. Furthermore, in mice injected with MN-EPPT, the reduction of T₂ decreased significantly after treatment, indicating a lower accumulation of MN-EPPT that reflected the reduced expression of uMUC-1 (9). To image epidermal growth factor receptor (EGFR) expression in gliomas, Shazeeb *et al.*, have devised a strategy of self-complementing enzymatic signal amplification, based on the specific local retention of paramagnetic products of di(tyramido)-DTPA(Gd) (diTyr-GdDTPA), a substrate of peroxidase (10). The study described a novel pretargeting approach that relies on the administration of an enzyme-linked anti-EGFR monoclonal antibody followed by the injection of a low molecular weight paramagnetic molecule, diTyr-GdDTPA, that is retained at the site of the anti-EGFR monoclonal antibody. In tumors pretreated with the specific targeted conjugates there was slower contrast agent elimination, compared to the non-pretreated tumors. This result was consistent with improved binding of the contrast agent to target cells in the presence of the targeted enzyme amplification pair, and suggested differences in substrate elimination from the tissue that was dependent upon the initial binding specificity of the monoclonal antibody (10).

Integrins and Angiogenesis

Angiogenesis is a critical element of tumor progression and invasion. The degree of neovascularization has been shown to correlate with tumor malignancy, and is an important factor in grading tumors in patients (11). Multiple MR probes have been developed to characterize tumor vasculature. Applications of DCE-MRI based on T₁ or T₂ changes induced by high or low molecular weight gadolinium-based paramagnetic contrast agents

have dominated the use of MRI in the assessment of vascular parameters, such as perfusion, vascular volume, and permeability surface area product (12). Recent studies have focused on developing agents that bind specifically to receptors expressed on tumor vasculature. Since the $\alpha_v\beta_3$ and $\alpha_5\beta_1$ members of the integrin family are closely associated with tumor angiogenesis, imaging probes that bind to these integrins can be used to assess tumor angiogenesis noninvasively *in vivo* (13, 14). The first *in vivo* use of $\alpha_v\beta_3$ -targeted MR nanoparticle to detect and characterize angiogenesis was described by Winter *et al.*, in a VX2 rabbit tumor model (15). The contrast agent injected was a lipid-encapsulated liquid perfluorocarbon (PFC) nanoparticle, characterized by a high target avidity and a prolonged systemic half-life. The nanoparticle carried a high level of paramagnetic ion payloads, linked to RGD (Arg-Gly-Asp) peptides that specifically bind to $\alpha_v\beta_3$ integrins on endothelial cells, and could be visualized in T_1 -weighted images (15). Ultrasmall superparamagnetic iron oxide (USPIO) particles linked to RGD peptides were also imaged *in vivo* with T_2^* -weighted MRI. The contrast generated was shown to be dependent on the level of $\alpha_v\beta_3$ integrin expression in a human squamous cell carcinoma xenograft models (16). Recently, the RGD peptide conjugated with high-density lipoprotein (HDL) nanoparticles has been used as a versatile and multimodal molecular imaging probe. HDL can be used as a multimodal nanoparticle platform for tumor vascular imaging *via* nonspecific accumulation and specific binding to angiogenically activated blood vessels (17). HDL nanoparticles carrying RGD peptide bind to angiogenic endothelial cells of tumors *in vivo* and can be visualized in T_1 -weighted images as shown in an Ewing's sarcoma tumor model (17).

In addition to RGD peptides that have been extensively used to image integrins *in vivo*, new targeting moieties are also being developed. For example, a novel non-RGD peptide (named P1c) derived from connective tissue growth factor can specifically bind to integrin $\alpha_v\beta_3$ (18). By linking the P1c peptide to USPIO, liver cancer cells expressing $\alpha_v\beta_3$ integrin were detected *in vivo* by MRI in an experimental model of liver cancer. The P1c-USPIOs specifically bound primary liver cancer cells that were imaged *in vitro* and *in vivo* (18). To improve tumor angiogenesis imaging, simultaneous targeting of two molecular markers of activated endothelium cells can also be performed. Paramagnetic liposomes labeled with gadolinium were directed against $\alpha_v\beta_3$ integrin and galectin-1 by attaching two ligands, angineX and cyclic RGD, on their surfaces (19). When the dual-targeted probes were imaged *in vivo* in B16F10 melanomas, they produced similar changes in MRI contrast parameters as the single-targeted liposomes. By using *ex vivo* fluorescence microscopy of tumor sections, a partial association of the liposomes with tumor endothelial cells was observed. Liposome accumulation was also found in the extravascular space of the tumor tissue. The extravascular accumulation was significantly less for dual-targeted liposomes compared to both single-targeted agents, showing that the dual-targeting approach significantly improved the specificity of the contrast agent for the tumor endothelium (19).

Cell tracking

Another application of MR probes has been in tracking the migration of stromal and immune cells to tumors, by pre-labeling them with gadolinium or iron-based contrast agents (20, 21). Examples of such studies include detecting the migration and homing of cells involved in tumor progression, such as fibroblasts, natural killer (NK) cells, or stem cells (20–22). Tumor-associated fibroblasts that play a critical role in tumor progression were prelabeled with two MR contrast agents, biotin-bovine serum albumin-Gd-DTPA or iron oxide particles, and MRI was used to visualize the injected fibroblasts noninvasively *in vivo* in an ovarian cancer model (20). Recruited fibroblasts co-localized with the neovasculature at the tumor rim, identifying their involvement in tumor angiogenesis (20).

Similarly, NK cells labeled with iron particles were imaged with MRI in a subcutaneous prostate cancer model. Accumulation of NK cells was visualized by MRI following a subcutaneous injection of labeled cells, and confirmed with histology. However, after an intravenous or an intraperitoneal injection of the NK cells, their migration in the tumor was confirmed by histology but could not be definitively detected by MRI. The lack of obvious changes in the MRI signal in tumors after intravenous or intraperitoneal injection of labeled cells, despite histologic evidence of their accumulation, could be due to the combination of a relatively low iron content per cell and a low density of cells in the tumors (22).

Bone marrow derived endothelial progenitor cells (EPCs) contribute to vasculogenesis and tumor growth. A subpopulation of human hematopoietic stem cells identified by the cell-surface molecule CD133 (AC133) has been shown to be more specific for endothelial differentiation and angiogenesis (21). *In vivo* tracking of EPCs prelabeled with SPIO demonstrated their migration, after local implantation to the periphery of the tumor, at the site of active angiogenesis (21).

An interesting property of stem cells (SCs) is their inherent tropism for primary and metastatic neoplasms in the adult brain. Consequently, they have been studied as promising vehicles to deliver therapeutic agents *in situ* (23). To optimize the development of SC-based therapies for the treatment of glioblastoma multiforme (GBM), Chaumeil *et al.*, labeled human mesenchymal SCs and fetal neural SCs with micron-size particles of iron oxide and imaged their migration towards two different orthotopic GBM models, circumscribed U87vIII, and highly infiltrative GBM26 (23). High resolution MRI was performed at 14.1T to monitor the *in vivo* location of the labeled SCs injected either in the contralateral hemisphere, or intratumorally in the tumor-bearing mice. Both types of SCs exhibited similar properties regarding tumor tropism and intratumoral dissemination, highlighting the potential of these SC sources as candidates for SC-based therapies (23). SCs can also be visualized *in vivo* using ^{19}F MRI. SCs readily internalize PFC nanoparticles and remain functional (24). PFC-labeled cells show distinct ^{19}F signals, enabling spatial cell localization with ^{19}F MRI (24).

^{19}F MRI has been used to track dendritic cells (DCs) labeled with perfluoropolyether agents and, combined with ^1H MRI, can be used to place the labeled cells in their anatomical context (25).

Tumor-associated macrophages (TAMs) are another essential component of the TME (26). The presence of TAMs during tumor growth can be assessed *in vivo* by injecting SPIO nanoparticles intravenously, as shown by Shih *et al* (27). Prelabeling is not necessary, since macrophages engulf these nanoparticles. Hypointense regions visible on T_2^* -weighted images indicated the presence of iron oxide particles that are mainly internalized by macrophages as a result of phagocytic activity, demonstrating the potential of MRI to monitor longitudinally the presence of TAMs with a single SPIO nanoparticle injection (27). SPIOs are, however, rapidly phagocytosed by macrophages in liver, spleen, and bone marrow, limiting their access to TAMs *in vivo*. USPIOs, on the other hand, are not as rapidly recognized by the reticulo-endothelial system, and consequently have a longer circulation time, allowing them to leak into tumor interstitium where they can be phagocytosed by TAMs (28).

PROBES FOR IMAGING THE PHYSIOLOGICAL ENVIRONMENT, THE EXTRACELLULAR MATRIX, AND METABOLISM

Hypoxia

The importance of hypoxia in cancer progression and treatment (29, 30) has resulted in the development of several noninvasive MR probes to detect tumor hypoxia. *In vivo* imaging of tumor oxygen consumption has been performed using ^{19}F MRSI of ^{19}F oxygen reporters. For instance, 2-nitro- α -[(2,2,2-trifluoroethoxy)methyl]-imidazole-1-ethanol (TF-MISO) is a nitroimidazole-based probe that accumulates in hypoxic cells through a biochemical reduction. The reduced probes bind to endogenous cellular molecules and accumulate in hypoxic cells (31, 32) and can be used to report on hypoxia *in vivo* with ^{19}F MRS (33). Hypoxic regions are identified by imaging the uptake, distribution and retention of the probes by acquiring ^{19}F MRSI (33). PFC can also be used to measure oxygen tension *in vivo* in tumor tissues with ^{19}F MRS, since its relaxivity is dependent on the concentration of dissolved molecular oxygen (34). It is also possible to assess oxygen tension in tissue with ^1H MRS after injection of the ^1H probe hexamethyldisulfoxane (HMDSO) since it has a single ^1H resonance with a spin-lattice relaxivity that is linearly dependent on pO_2 (35).

Blood oxygenation level dependent (BOLD) contrast (36), has been applied to characterize tumor vasculature using the endogenous contrast produced by deoxyhemoglobin (37). The presence of deoxyhemoglobin causes a susceptibility difference between the blood vessel and the surrounding tissue, inducing microscopic magnetic field gradients that can be measured in T_2^* -weighted images. Oxyhemoglobin is diamagnetic, and does not produce the same dephasing, as a result, changes in blood oxygenation can be observable as signal changes in T_2^* -weighted images (12). This approach provides a noninvasive measurement of tumor fractional blood volume without requiring the administration of an exogenous contrast, and works best in poorly oxygenated tumors and human xenografts. BOLD image contrast has some limitations, since its relationship with angiogenesis is complex, and not solely determined by the oxygenation-status of the blood. It is also affected by other factors such as oxygen saturation, hematocrit, blood flow, blood volume, vessel orientation and geometry. In a comparison of BOLD imaging with ^{19}F MRI to measure tumor oxygenation, a strong correlation was observed between the BOLD signal response and changes in pO_2 in rat breast 13762NF carcinomas (38).

pH

MRS measurements of tumor pH have played an important role in identifying the acidic extracellular and neutral to alkaline intracellular environments in cancer cells and tumors (39, 40). The high glycolytic activity of tumors combined with chaotic tumor vasculature creates acidic extracellular microenvironments in tumors that influence tumor progression, metastasis and drug resistance (41–43). The chemical shift of the inorganic phosphate (Pi) signal detected in ^{31}P MR spectra is pH-dependent, and since Pi is primarily intracellular, reports on intracellular pH (pHi) (44). Tumor pHi measured by ^{31}P MR is typically neutral to alkaline. In contrast, acidic pHe values were detected using an exogenous ^{31}P -detectable pH marker 3-aminopropylphosphonate (3-APP) (45). Intra- and extracellular pH can be measured simultaneously from the chemical shifts of exogenous 3-APP and endogenous inorganic phosphate, respectively, as shown by Robey *et al.* (Figure 2A) (39), although the low sensitivity of ^{31}P MRS results in limited spatial resolution (46). The study showed that NaHCO_3 treatment increased pHe, resulting in a reduced incidence of metastases from MDA-MB-231 tumor xenografts (39). To improve spatial resolution, pHe imaging can be performed in tumors *in vivo* using probes detected with ^1H MRSI such as (imidazol-1-yl)3-ethoxycarbonylpropionic acid (IEPA) (45), or 2-(imidazol-1-yl)succinic acid (ISUCA) (47).

Relaxation enhanced pH measurements can be performed *in vivo* by using gadolinium-based contrast reagents with a pH-dependent relaxivity pH, such as Gd-DOTA-4AmP⁵⁻ (48). Since these relaxation-based pH agents are also concentration dependent, analogous pH-independent probes such as Gd-DOTP⁵⁻ can be injected in the same animals to correct for delivery. The dual injection allows analysis of tumor perfusion and pHe. As shown in Figure 2B, an inverse correlation of the two parameters was observed in Time to Maximal Intensity (TMI) maps of both contrast agents, and the related pHe maps acquired on a well-perfused glioma model (48), identifying poor vascularization as a major contributor for the acidic pHe observed in tumors. pH sensitive chemical-exchange-dependent saturation transfer (CEST) agents have also been developed for noninvasive MRI, as discussed elsewhere in this issue.

Proteolytic activity

Several proteases are involved in the process of invasion and metastases, as they rearrange and degrade components of the ECM. Amongst these, matrix metalloproteinases (MMPs) play a key role (49). Targeted probes developed to detect MMPs activity have mostly been PET and optical-imaging based (50). More recently, several new approaches have been explored to image MMP activity *in vivo* with MRI (51, 52). Olson *et al.*, have described activatable cell penetrating peptides (ACPPs) linked to dendrimeric nanoparticles labeled with gadolinium, as probes for *in vivo* MR imaging of proteases, especially MMP-2 and MMP-9 (52). ACPPs are short polycations attached *via* protease-cleavable linkers to neutralizing polyanions. The polyanion masks the adhesiveness of the polycation until a protease cuts the linker. The polycation is released by protease activity, and the associated cargo can then adhere to and be taken up within cells in the immediate vicinity of the protease (52). Dendrimers labeled with Gd-DOTA moieties combined with ACPPs injected into HT-1080 tumor bearing mice were shown to accumulate in xenografts by a protease-dependent mechanism. The study revealed that the most intense ACPP dendrimers uptake, visible in MR T₁-weighted images, correlated with high MMP activity at the invasive edge of the tumor (52).

Transglutaminases are also involved in tumor progression and invasion since they mediate tissue remodeling by catalyzing covalent cross-links between proteins of the ECM (53). Tissue transglutaminase activity has been recently assessed *in vivo* with MRI by using an imaging probe composed of a peptide DCCP16, extracted from α 2-antiplasmin, the primary inhibitor of plasmin-mediated fibrinolysis. The peptide was linked to an imaging reporter labeled with Gd-DOTA that was visible on T₁-weighted images (53). The probe was tested *in vivo* in a model of 4T1 mammary gland tumor, 24 h after injection, retention of the transglutaminase-targeted probe was observed in the tumor, that was not the case for the negative control probe. The peptidic moiety present on the probe served as a transglutaminase substrate analog, and thus allowed the binding of the probe on transglutaminase present in the tumor, allowing its detection by MRI. Noninvasive imaging of transglutaminase activity by MRI can provide an important tool for monitoring transglutaminase-targeted therapy (53).

¹³C MRS and hyperpolarization

The use of ¹³C labeled substrates has been applied for several decades to understand metabolic flux through various pathways (54–56). Recent developments in hyperpolarization have provided a significant advantage in sensitivity that overcomes low sensitivity limitations of ¹³C MRS (57). The hyperpolarization of spins, described in detail in a separate article in this issue, increases the sensitivity of detection of the MR signal of ¹³C-labeled substrates by >10,000. Hyperpolarized ¹³C-labeled substrates can be injected systemically, allowing real-time metabolic mapping (58–60). Hyperpolarized ¹³C has been

applied to measure *in vivo* pHe, and study several metabolic pathways and enzymatic activity by labeling different substrates such as pyruvate, glutamate, or fumarate (61–64). Since the hyperpolarized magnetization decays with time, substrates with T_1 's in excess of 20 s are necessary to image metabolic events, imposing limitations on available probes (57).

CEST

In vivo probes can also be detected by contrast generated using magnetization transfer, a frequency-selective MR technique used to evaluate ^1H and other nuclei chemical exchange (65). Also described in a separate article in this issue, proton CEST can be used for several applications such as measuring pH *in vivo*, detecting exogenous CEST-contrast agents, or detecting amide protons of endogenous mobile cellular proteins and peptides, (66, 67). Similar to fluorescent proteins in optical imaging, CEST reporter proteins can be used for molecular imaging studies in gene promoter and expression studies (68).

METASTASES DETECTION

In addition to identifying proteolytic activity in the ECM, MRI probes are also making inroads into understanding the metastatic cascade (2), and in detecting small nodules in patients. Diffusion-weighted (DW) MRI can be applied to detect metastases *in vivo*, as shown by Lin *et al.*, in a clinical study where the detection of pelvic lymph nodes was improved by combining size analysis on anatomic images with apparent diffusion coefficient (ADC) values, reaching a sensitivity of 83% and a specificity of 99% (69). Specific MR sequences, such as DW whole-body imaging with background body signal suppression (DWIBS) (70, 71), have been developed to acquire 3D-DW whole body images to screen for metastases. Other methods of metastases detection involve the administration of specific probes with MR contrast. Amongst these, SPIO particles that create negative contrast visible in T_2 and T_2^* -weighted images, can be used to detect metastatic nodules *in vivo* with MRI. Early clinical use at 1.5T of superparamagnetic ferrite particles to detect metastasis did not show significantly more metastases in contrast-enhanced images compared to regular T_2 -weighted images (72). However, in a preclinical study, lymph node metastases were detected after interstitial administration of a SPIO contrast agent, AMI-25. The signal intensity of normal lymph nodes decreased significantly after interstitial administration of AMI-25, whereas lymph nodes with metastases showed no significant change in signal intensity (73). More recently, lymph node metastases have been detected in prostate cancer patients using highly lymphotropic superparamagnetic particles injected intravenously. The particles localize in the lymph nodes through interstitial-lymphatic fluid transport, and an abnormal pattern of SPIO accumulation occurs in the presence of cancer cells in the lymph nodes that can be detected by MRI with the acquisition of T_2^* -weighted images (74). In another clinical study, lymphotropic nanoparticle enhanced MRI of prostate cancer patients who underwent radical prostatectomy identified occult lymph node metastases, that were not enlarged based on size criteria (75). Gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA) is another MR probe that can be used to detect and characterize focal hepatic lesion. It is a liver-specific hepatobiliary contrast agent that can effectively be used in dynamic and static MRI of hepatocytes to achieve accurate delineation, classification, and characterization of liver tumors (76, 77). When combined with diffusion-weighted images (DWI), it allows an improvement of the detection of small liver metastases, as illustrated in Figure 3, that demonstrates the confirmation of a second lesion using DWI (77).

THERAPY AND THERANOSTICS

Two major problems in cancer treatment are the uncertainty of drug delivery because of the chaotic characteristics of tumor vasculature (42), and the ability to deliver therapeutic agents

selectively to cancer cells without damaging normal tissues and causing major collateral damage. MR probes can play a role in evaluating both these aspects. For instance, *in vivo* detection and localization of anticancer ^{13}C labeled-drugs can be performed with MRS (78). MRSI of the intratumoral distribution of the ^{13}C -labeled anticancer agent temozolomide demonstrated the heterogeneous delivery of the drug, which is used clinically to treat glioblastoma and anaplastic astrocytoma (78). Emerging ^{13}C hyperpolarization approaches may provide significant improvement in sensitivity for such applications.

Recently Kato and Artemov (79) have described a novel dual contrast MRI technique that detects release of agents from nanocarriers using simultaneous encapsulation of SPIO nanoparticles and a Gd-based paramagnetic contrast agent. Since SPIO and GdDTPA bismethylamide (GdDTPA-BMA) have very different diffusion properties, when the nanocarrier is intact, the SPIO and GdDTPA-BMA are in close proximity, and a strong negative signal enhancement due to the T_2 effects of SPIO dominates the positive T_1 contrast generated by GdDTPA-BMA. A positive T_1 contrast is observed once the distance between GdDTPA-BMA and SPIO molecules is beyond the T_2 enhancement range when the nanocarrier releases its cargo, providing information on the delivery of the nanocarrier and the release of its cargo.

Magnetic nanoparticles that contain an iron oxide magnetic core coated with oleic acid and stabilized with a pluronic copolymer have been shown to provide sustained delivery in a MCF-7 breast cancer xenograft *in vivo* (80), and can be developed as effective theranostic agents to target cell surface receptors, and deliver anticancer agents (80).

Recent integrations of MRI with other molecular imaging techniques, such as optical and PET imaging, have shown promise in further characterization of tumor, and validation of drug delivery. Kircher *et al.*, (81) have shown a more accurate delineation of the margins of brain tumors in living mouse both preoperatively and intraoperatively using a triple modality MRI-photoacoustic-Raman imaging nanoparticle.

PET/NIR fluorescent/MRI triple iron oxide nanoparticles have also been developed, as demonstrated in a recent study where dopamine-nanoconjugates were encapsulated in human serum albumin matrices. These matrices are clinically utilized as drug carriers (82). The nanoparticles were labeled with ^{64}Cu -DOTA for PET imaging, Cy5.5 for optical imaging and the iron core provided MRI contrast. *In vivo* these nanoparticles provided a prolonged circulation half-life, significant accumulation in U87MG tumors, a high extravasation rate, and low uptake by macrophages in the tumor region (82).

MRI can not only be used to monitor the relative accumulation of labeled-drug nanocarriers in tumor tissue, but also to select an optimal treatment time course, and evaluate therapeutic response and efficacy of treatment strategies. Prodrug enzyme therapies have been developed to minimize damage to normal tissue by delivering an activating enzyme that localizes specifically in the tumor and then converts a non-toxic prodrug to a cytotoxic agent locally in the tumor. Imaging the delivery of the enzyme within the tumor is crucial to time the administration of the prodrug, and to avoid damage to normal tissue. MR techniques can be applied to assess not only the delivery of the enzyme, but also the efficacy of the treatment (83, 84). In order to amplify selective targeting of cancer cells while sparing normal tissue, it is possible to combine small interfering RNA (siRNA)-mediated silencing of cancer cell-specific targets with the delivery of a prodrug enzyme. MRI can then be applied to determine effective tumor delivery of the siRNA and the prodrug enzyme to time the prodrug administration, to detect target down-regulation by siRNA, and to confirm the conversion of the prodrug by the enzyme (Figure 4). An MR contrast agent, such as gadolinium, can be used to label the probe that carries the prodrug enzyme. The prodrug

enzyme used can be, for example, bacterial cytosine deaminase (bCD) that converts the nontoxic prodrug 5-fluorocytosine (5-FC) to cytotoxic 5-fluorouracil (5-FU). As shown in Figure 4, the siRNA downregulated choline kinase (Chk), an enzyme significantly up-regulated in aggressive breast cancer cells. *In vivo* MRI showed efficient intratumoral probe delivery (Figures 4A and B). The siRNA-mediated downregulation of Chk and the conversion of 5-FC to 5-FU by bCD were measured noninvasively with ^1H MRSI and ^{19}F MRS respectively (Figures 4C and D). The study showed the advantages of combining MRI, MRS and MRSI to assess and optimize the efficacy of siRNA and enzyme prodrug therapy.

MRI probes have also been used *in vivo* to visualize delivery of molecular reagents, such as siRNA (84, 85) in the exciting new area of theranostic imaging where therapeutic cargo is delivered to cancer-specific targets that can be noninvasively imaged. Kumar *et al.*, have described a magnetic nanoparticle-labeled probe targeting tumor specific uMUC-1 receptor that delivers siRNA directed against the anti-apoptotic gene *birc5*, which encodes the protein survivin (85). The delivery of the probe in subcutaneous breast tumor xenograft was monitored *in vivo* by acquiring T_2 -weighted images, as illustrated in Figure 5. Effective delivery of the probe induced a loss of signal in the T_2 -weighted images after injection of the nanoparticle. The decrease of tumor T_2 relaxation could be quantified, as shown in Figure 5. Treatment with the probe resulted in a 5-fold increase of apoptosis in the tumors treated with the anti-*birc5* siRNA compared to the ones treated with the scrambled siRNA (85).

Specific targeting of theranostic agents can improve their delivery, as shown by Schmieder *et al* (13). A theranostic agent was created by incorporating the anti-angiogenic agent fumagillin to a dual $\alpha_5\beta_1(\alpha_v\beta_3)$ -targeted nanoparticle, loaded with gadolinium to be visible with MRI. The anti-angiogenic effect of the agent was demonstrated with 3D MRI in an MDA-MB-435 breast cancer xenograft model (13). $\alpha_5\beta_1$ integrin, poorly expressed on normal quiescent vessels, is highly expressed on tumor blood vessels, and as a consequence, is a good target for theranostic agents. The injection of the nanoparticles resulted in a decrease of the tumor neovasculature, without affecting the tumor size, showing that the particles can be used to deliver a therapeutic cargo, and to detect the efficacy of the treatment, by reporting on the spatial organization of tumor vessels (13). In another study, VX2 tumor-bearing rabbits were treated on days 6, 9, and 12 post-implantation with gadolinium labeled $\alpha_v\beta_3$ -targeted fumagillin nanoparticles and imaged on day 16 (86). Quantitative MRI revealed that the neovasculature was predominantly located in the periphery of the tumor. Treatment with targeted nanoparticles reduced neovascularization and decreased tumor volumes in treated rabbits, compared to the control groups (86).

While receptors, antigens and integrins provide viable targets for cancer-specific delivery of theranostic probes, many cancer cells do not express specific receptors or antigen, and as a result it is becoming increasingly important to mine metabolism, angiogenesis, inflammation, the TME, and stromal cell receptors for tumor specific delivery for image-guided targeted molecular medicine and examples of exploiting the TME for theranostic imaging were recently reviewed by Stasinopoulos *et al* (1).

CONCLUSION

Smart MR probes investigating many of the complex aspects of cancer are available for detection, diagnosis, and prognosis, and equally importantly for understanding cancer at the bench. The future of effective cancer treatment lies in modifying and adapting treatments to match pathways and targets identified genomic and proteomic analysis of individual tumors that will certainly benefit from the development of novel highly sensitive and specific methods for noninvasive molecular imaging of cancer. MRI and MRS in combination with

novel MR probes should play a major role in characterizing individual tumors and designing effective treatments. However, unlike nuclear imaging that has nanomolar to picomolar sensitivity, MRI and MRS have the drawback of sensitivity in the micromolar range, and amplification strategies such as hyperpolarization or probes that allow combined multi-modal imaging with PET or SPECT are very likely going to be future areas of emphasis. The limited sensitivity and the resulting need for high concentrations of contrast agents continue to pose a challenge in terms of FDA approval for clinical applications, as do nanoparticles, particularly multifunctional nanoparticles. Quantitation and reproducibility of probe design are other areas that will require emphasis for the MR probes to successfully translate into making significant advances in cancer treatment and survival.

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ABBREVIATIONS

5-FC	5-fluorocytosine
5-FU	5-fluorouracil
ACPPs	activatable cell penetrating peptides
ADC	apparent diffusion coefficient
bCD	bacterial cytosine deaminase
BOLD	blood oxygenation level dependent
CEST	chemical-exchange-dependent saturation transfer
Chk	choline kinase
CT	computed tomography
DC	dendritic cells
DCE	dynamic contrast enhanced
DW	diffusion-weighted/
DWI	diffusion-weighted images
ECM	extracellular matrix
EGFR	epidermal growth factor receptor
EPC	endothelial progenitor cell
ER	estrogen receptor
GBM	glioblastoma multiformae
GdDTPA	gadolinium-diethyltriaminepentaacetic acid
GdDTPA-BMA	gadolinium-diethyltriaminepentaacetic acid bismethylamide
HDL	high-density lipoprotein
MMP	matrix metalloproteinase
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy

MRSI	magnetic resonance spectroscopic imaging
NIR	near infrared
NK	natural killer
PET	positron emission tomography
pHe	extracellular pH
pHi	intracellular pH
Pi	inorganic phosphate
SC	stem cell
siRNA	small interfering RNA
SPECT	single photon emission computed tomography
SPIO	superparamagnetic iron oxide
TAM	tumor-associated macrophage
TME	tumor microenvironment
TMI	time to maximal intensity
PFC	perfluorocarbon
USPIO	ultrasmall superparamagnetic iron oxide

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Figure 1.
Schematic of the applications of MR probes in imaging and treating cancer.

Figure 2.

(A) *In vivo* image-guided volume-selective ^{31}P MR spectra acquired on a 4.7T spectrometer from control (solid) and NaHCO_3^- treated (dotted) MDA-MB-231 tumor xenografts. 3-APP, 3-aminopropylphosphonate; P_i , endogenous inorganic phosphate; PME, phosphomonoesters; NTP, nucleoside triphosphate. Inset represents the average values for tumor pH_i and pH_e in the absence and presence of bicarbonate treatment (columns) and SE (bars). Adapted with permission from Robey *et al.* (39). (B) Representative Time to Maximal Intensity (TMI) and pH_e maps in C6 glioma *in vivo*. Upper and lower panels correspond to 2 different animals. (1) T₁-weighted images, (2) TMI maps of Gd DOTP⁵⁻, (3) TMI maps of GdDOTA⁻⁴AmP⁵⁻, (4) pH_e maps. Adapted with permission from Garcia-Martin *et al* (48).

Figure 3.

Surgically confirmed liver metastasis from a rectal carcinoma in a 59-year-old man. (A) Axial portal venous phase and (B) 20-minute hepatobiliary phase image after injection of gadoxetic acid, only a large mass (arrows) was interpreted as a metastasis (score 4). An additional tiny lesion (small arrow) was missed by both observers during image interpretation. (C) Axial single-shot echo-planar diffusion-weighted imaging ($b = 800 \text{ s/mm}^2$), 2 lesions are clearly seen as bright hyperintense (arrows). (D) Corresponding apparent diffusion coefficient map, 2 lesions are visually lower than adjacent liver (arrows). Adapted with permission from Kim *et al.* (77)

Figure 4.

(A) Representative *in vivo* T₁-weighted MR images (top panel) and quantitative T₁ maps (bottom panel) of a tumor pre- and post-injection of nanoplex. (B) Time-dependent mean T₁ values of tumors pre- and post-injection of nanoplex. (C) *In vivo* ¹⁹F MRS demonstrated efficient conversion of prodrug 5-FC to 5-FU and its metabolites by nanoplex localized in the tumor. (D) Representative *in vivo* tCho maps and color-coded tCho intensity maps overlaid on corresponding T₁-weighted images of a tumor before, 24 and 48 h after nanoplex injection. Adapted with permission from *Li et al.* (84)

Figure 5.

In vivo MRI in a subcutaneous breast tumor model. (A) Representative precontrast and postcontrast T₂-weighted images (top) and color-coded T₂ maps (bottom) of tumor-bearing mice injected *i.v.* with MN-EPPT-siBIRC5 (10 mg/kg iron). The tumors (outlined) were characteristically bright (T₂ long) before contrast. At 24 h after injection, there was a loss of signal intensity (T₂ shortening) associated with the tumors, indicative of nanodrug accumulation. (B) Quantitative analysis of tumor T₂ relaxation times. T₂ map analysis revealed a marked shortening of tumor T₂ relaxation times 24 h after nanodrug injection, indicating accumulation of MN-EPPT-siBIRC5. Adapted with permission from *Kumar et al.* (85)