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Applications of molecular MRI and optical imaging in cancer

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Abstract

Some of the most exciting advances in molecular-functional imaging of cancer are occurring at the interface between chemistry and imaging. Several of these advances have occurred through the development of novel imaging probes that report on molecular pathways, the tumor micro-environment and the response of tumors to treatment; as well as through novel image-guided platforms such as nanoparticles and nanovesicles that deliver therapeutic agents against specific targets and pathways. Cancer cells have a remarkable ability to evade destruction despite the armamentarium of drugs currently available. While these drugs can destroy cancer cells, normal tissue toxicity is a major limiting factor, a problem further compounded by poor drug delivery. One major challenge for chemistry continues to be to eliminate cancer cells without damaging normal tissues. Here we have selected examples of MRI and optical imaging, to demonstrate how integrating imaging with novel probes can facilitate the successful treatment of this multifaceted disease.

Challenges in cancer

The characteristics that confer lethality to cancer are uncontrolled proliferation, the ability to establish a vascular network, to invade and metastasize and to induce cachexia [1]. The unique physiological environments in tumors (e.g., hypoxia) that result in resistance to radiation and chemotherapy, the evolution of transporters that effectively pump out therapeutic agents and poor delivery, due to the inadequate vasculature found in tumors, contribute to the ability of cancer cells to adapt and survive treatment. In addition, cancer cells can invade through the extracellular matrix (ECM) of the primary tumor and metastasize to distant sites. Metastasis-related organ failure is one of the main causes of mortality from cancer [2]. Targeting pathways that are critical for cancer cell proliferation, including angiogenesis, invasion and metastasis are therefore being actively investigated in cancer treatment and, by using novel probes, imaging is making significant inroads in identifying such pathways as well as in detecting the response to such targeting. Molecular imaging uses modalities such as computed tomography (CT), MRI, ultrasound and nuclear-imaging techniques such as positron emission tomography (PET) and single-photon emission tomography (SPECT). The study of cancer encompasses many disciplines from the basic sciences, which explore the genetic and biochemical origins and progression of neoplastic disease, to clinical practice, where cancer detection and treatment

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take place. Molecular imaging is being incorporated in virtually all of these areas, in applications that span basic and preclinical research to the clinical management of cancer.

In this review, we have focused on examples that incorporate MRI and optical imaging. In the following section we provide brief overviews of these imaging modalities. Some of the advantages and limitations of these techniques are listed in Table 1.

Positron emission tomography and SPECT imaging, two major modalities used for molecular imaging, are not discussed here, but relevant reviews describing the applications of these modalities are outlined. These nuclear-imaging techniques require the administration of radiotracers that are taken up by the body, the γ -rays emitted by the radiotracers are then detected. Nuclear-imaging techniques are sensitive and able to detect pico- to femto-molar concentrations of radiotracer quantitatively. Both SPECT and PET are inherently tomographic but have spatial resolutions of approximately 5 mm for clinical scanners and approximately 0.5–1 mm for small animal scanners. Usually the high spatial resolution of colocalized CT images is used to provide the anatomic details in SPECT or PET images. Several PET and SPECT agents have been translated for clinical imaging [3–9]. These include radiopharmaceuticals that target receptors in angiogenesis, steroid hormone receptors, as well as various growth receptors [10,11] and radiopharmaceuticals for measuring metabolism, proliferation and hypoxia [5,12]. PET and SPECT agents are also being incorporated in monitoring gene therapy [13].

MRI & optical imaging

With its noninvasive characteristics and exquisite spatial resolution, MRI is one of the most powerful imaging techniques available in diagnostic imaging and preclinical results can be translated with relative ease to the clinic. Recent advances in the development of molecular targeted contrast agents have expanded the traditional strengths of MRI and magnetic resonance spectroscopy (MRS) of characterizing functional tumor parameters such as pH, vascularization, metabolism [14] and cell death, to include visualization of molecular pathways [15].

Since nuclei with nonzero magnetic moments precess with a magnetic resonance frequency of ω_0 in the presence of an external static magnetic field, B_0 , it is possible to detect the radiofrequency (RF) signals generated by these precessing spins, by applying a RF pulse at the magnetic resonance frequency. The magnetic resonance frequency ω_0 is linearly dependent on B_0 and on the gyromagnetic ratio of the nucleus γ , as $\omega_0 = \gamma B_0$ and the magnetic resonance signal intensity is dependent on the concentration and the gyromagnetic ratio γ of the nuclear spins. Two rate constants, the spin–lattice (or longitudinal relaxation time) T_1 and the spin–spin (or transverse relaxation time) T_2 , govern the time dependence of the magnetization signal.

By applying magnetic field gradients, the proton signal from the abundant water in tissues is spatially encoded to obtain magnetic resonance images with a high spatial resolution of approximately 100 μm . Most contrast agents or reporters used in MRI are detected by virtue of the changes they induce in the T_1 or T_2 of tissue water. T_1 contrast agents result in an enhancement or brightening of signal in a T_1 -weighted image while T_2 contrast agents result in a loss of signal in a T_2 -weighted image.

Magnetic resonance spectroscopy provides information about the chemical environment of nuclei such as the number of chemical bonds, neighboring nuclei and chemical structure. The chemical shift of each peak in an magnetic resonance spectrum depends on the chemical structure of the metabolite or compound, with the peak area being proportional to the concentration of the metabolite. magnetic resonance spectra can be acquired without any spatial information (unlocalized) or localized to specific volumes. In MRS imaging (MRSI), chemical

information is spatially encoded and the localized spectra are processed to obtain images of metabolites or exogenous substances.

MRI methods are noninvasive and, therefore, can be easily translated from preclinical research to clinical applications, allowing the acquisition of spatial as well as temporal information. However, MRI suffers from less than optimum sensitivity of detection of injected contrast agents. That same lack of sensitivity also applies to MRS and MRSI techniques that provide a wealth of functional information, but which are limited by the sensitivity of detection. Therefore, increasingly in preclinical discovery studies, and as outlined in some of the examples shown here, the sensitivity of optical imaging is being combined with MRI or MRSI for applications in cancer research.

Optical imaging techniques have traditionally played an important role in molecular imaging of gene reporters, molecular targets and receptors in preclinical studies of cancer [16]. The availability of novel optical imaging contrast agents and tomographic techniques have further advanced the use of optical imaging in these studies [16–18]. Optical reporters are typically either bioluminescent or fluorescent based and are used either as gene reporters or covalently linked to delivery vehicles. Firefly and renilla luciferases are the most commonly used bioluminescent reporters. In bioluminescent reporter systems, luciferase acts on its systemically administered substrate luciferin, resulting in bioluminescence. Typically, a reporter gene expressing luciferase is used to detect cells expressing that gene *in vivo*.

Fluorescent proteins such as GFP or red fluorescent protein are used as fluorescent optical reporter-based gene reporter systems. Genetically modified proteins such as mBanana, tdTomato and mCherry are now available and allow the use of multiple gene reporters in cells [19].

A recent study has described a protein known as Katushka and its monomer mKate, with excitation at 588 nm and emission at 635 nm [20]. This far-red emission wavelength is beyond the absorbance of hemoglobin and melanin at 620 nm and below the absorbance of water at 1100 nm and therefore provides greater penetration depth for detection *in vivo* [20].

Novel optical contrast agents for sentinel lymph node identification using optical imaging are also being developed [21]. Quantum dots or other tracers that fluoresce in the near-infrared region are injected into the esophageal wall and migrate to sentinel lymph nodes where they can be detected. In addition, nonlinear techniques such as second harmonic generated (SHG) microscopy can be used for the evaluation of the collagen structure of tissue [22]. SHG is a second-order nonlinear optical process that arises from a medium lacking a center of symmetry [23]. Upon illumination of materials that lack inversion symmetry, the nonvanishing second-order susceptibility results in the generation of an optical field at exactly twice the frequency (half the wavelength) of the incident light [24]. Since SHG is based entirely on endogenous molecular properties, it does not involve direct electronic excitation, avoiding phototoxicity and photo-bleaching of dye molecules. Fibrillar collagen is the most widely studied biomolecular source of SHG [24]. Brown *et al.* showed that a highly fibrillar subpopulation of collagen-I was the primary source of the second harmonic signal from tumors implanted in immunodeficient mice [25].

Owing to limited depth of penetration, clinical applications of optical imaging to detect malignant lesions have been primarily endoscopic or laparoscopic. Optical coherence tomography (OCT) and optical Doppler tomography (ODT) are two techniques that are being explored for minimally invasive imaging of near-surface abnormalities [26]. OCT allows macroscopic characterization of epithelial and subepithelial structures at spatial resolutions of 2–20 μm at a depth of approximately 2 mm, with catheter probes that are typically 2–3 mm in diameter [26–28]. OCT has been combined with ODT to provide high-resolution images of

static and moving components such as local blood flow velocities, and preclinical studies have demonstrated its utility in cancer detection [26,29].

Imaging receptor & protein expression

As cancer is such a complex disease, it is important to be able to tailor drugs to match the profile of individual tumors through ‘personalized treatment’. Noninvasive MRI and MRSI have the potential to fill an important niche in personalized treatment through imaging receptor and protein expression. The low concentration of receptors and molecular targets and the inherent insensitivity of magnetic resonance detection do, however, impose limitations on the clinical translation of some of the preclinical studies presented here and will require amplification strategies to increase the sensitivity of detection. However, this ability is important in preclinical drug discovery and several preclinical MRI studies have used antibodies to target antigens overexpressed by tumor cells, such as c-Met for gliomas [30] and HER2/neu for breast cancer [31]. Magnetic resonance contrast was generated by using a biotinylated antibody that binds to the receptor, followed by administration of avidin linked to gadolinium-diethylenetriaminepentaacetate (GdDTPA) [31] or by direct binding of GdDTPA to the antibody through bovine serum albumin [30]. In the latter study, biotin was incorporated into the contrast agent to relate the presence of contrast agent to receptor expression in histological sections.

Peptides have also been used for tumor-specific labeling [32,33]. The C3d peptide binds to neural cell adhesion molecule, which is stably expressed by the tumor endothelial cells [32]. This C3d peptide was conjugated with apoferritin loaded with Gd-HPDO₃A, which provided sufficient sensitivity to allow *in vivo* MRI visualization of tumor-derived endothelial cells [32].

A class of magnetic resonance contrast agents, based on proton chemical exchange-dependent saturation transfer (CEST), is also being developed for molecular MRI. CEST contrast is achieved by selectively irradiating water-exchangeable protons and detecting the effect of the CEST on the water signal [34]. This approach was used to detect a lysine-rich protein reporter in cells genetically engineered to express lysine-rich protein. Different polypeptides with different resonance frequencies of the exchangeable protons can provide multiple CEST reporters to detect multiple targets within the same magnetic resonance image [35]. The sensitivity of detection of CEST (~50 mM concentration) can be increased by using paramagnetic lanthanide chelates (PARACEST) that improve the sensitivity of detection of the contrast agent to approximately 10 mM [36].

Molecular imaging of the expression of serine proteases such as hepsin has also been performed. Hepsin is a serine protease that is expressed in prostate cancer, but is absent or present at low concentrations in normal prostate. Human prostate cancer xenografts were imaged *in vivo* in mice following injection of a hepsin-targeted imaging agent (Figure 1) [37]. Fluorescent nanoparticles were conjugated to a peptide directed specifically against hepsin and injected intravenously in tumor-bearing mice. Visualization of an approximately 5-mm prostate tumor xenograft was possible with tomographic optical imaging [37].

Imaging the ECM, protease activity & invasion, angiogenesis & metastasis

The ECM, invasion & protease activity

Cancer cells have the ability to invade through the ECM and metastasize to distant sites. Invasion through the ECM is achieved by the secretion of several degradative enzymes such as metalloproteinases, plasminogen activators, cathepsins and hyaluronidase [38–40]. As a result, developing proteolytic enzyme inhibitors is an active area of research and the ability to

non-invasively detect proteolytic enzyme activity would be valuable in selecting tumors for specific inhibitors and for detecting response to such agents.

Recently, transglutaminase activity was detected in preclinical studies with MRI using contrast generated by a peptide with a specific recognition sequence for transglutaminase linked to GdDTPA. Hyaluronidase activity was detected by a similar approach by using a contrast agent that combines hyaluronan with GdDTPA and nontoxic agarose beads. Transglutaminase activity generated MRI contrast by altering the relaxation rate of water molecules in contact with the contrast agent [41]. The same mechanism was exploited to detect hyaluronidase activity [42]. Hyaluronidase activity was studied noninvasively both in cell culture and in an ovarian cancer xenograft model by changes induced by the contrast agent in the relaxation rate of water [42].

Fluorescence molecular tomography (FMT) is capable of imaging tissues at depths of less than 10 cm by reconstructing the *in vivo* distribution of injected far-red and near-infrared fluorescent (NIRF) probes [43,44]. FMT utilizes mathematical models describing photon propagation in tissues to allow 3D reconstruction of fluorescence in live animals [45]. To perform fluorescence tomography, the tissue is illuminated at different projections and multiple measurements are collected from the boundary of the tissue of interest [46]. FMT produces quantified 3D reconstructions of fluorescence activation or concentration, using measurements of fluorescent molecular probes at both the emission and excitation wavelengths. One of the advantages of FMT is that it does not require any measurements obtained before contrast agent injection [46]. This technique was used to image cathepsin cysteine protease activity in 0.93–1.3-mm sized lung tumors in mice, using optical sensor probes activated by cathepsin cysteine proteases [44].

Since proteolytic enzymes degrade the ECM, the integrity of the ECM can also be used to characterize the invasiveness of the tumor and potentially design treatments that may alter ECM integrity for improving drug delivery to the tumor interstitium. Characterization of the extravascular transport of macromolecules using MRI through the ECM of solid tumors *in vivo* can provide an index of ECM integrity [47,48]. Macromolecular MRI of two human breast cancer models with significantly different invasiveness was used to quantify and understand the role of interstitial fluid transport, lymphatic-convective drain and vascularization in axillary lymph node metastasis. Results from this study suggested that increased lymph node metastasis may occur due to a combination of increased invasiveness and reduced ECM integrity, allowing increased pathways of least resistance for the transport of extravascular fluid, as well as tumor cells [47].

Angiogenesis/vascularization

MRI methods for deriving perfusion parameters are uniquely suitable for characterizing tumor vasculature. The only class of paramagnetic contrast agents in clinical use is the low-molecular weight Gd-based agents. For preclinical models, a wide variety of contrast agents, macromolecular as well as targeted, are available to characterize tumor vasculature [49].

Although the low-molecular weight Gd-based agents do not cross the cell membrane and are not considered freely diffusible, they can be used to derive tracer kinetic parameters such as K_{trans} (min^{-1}), the volume transfer constant between the blood plasma and the extravascular extracellular space (EES), k_{ep} (min^{-1}), the rate constant between the EES and blood plasma and v_e (%), the volume of the EES per unit volume of tissue [50,51].

A combined MRI and optical imaging approach using human prostate cancer xenografts engineered to express GFP under hypoxia, was used to understand the dynamics between tumor vascularization and hypoxia using MRI of a macromolecular contrast agent albumin-GdDTPA

[52]. MRI was used to obtain colocalized maps of vascular volume and permeability, while optical imaging was used to obtain colocalized maps of hypoxia. These studies were performed using PC-3 human prostate cancer xenografts stably expressing GFP under control of a hypoxia-response element. Coregistered maps of vascular volume, permeability and GFP demonstrated that hypoxic regions were characterized by low vascular volume and frequently by high permeability because hypoxia increased the expression of the potent permeability factor VEGF [52]. In the context of drug delivery, these data suggest that large macromolecular therapeutic agents are likely to leak out more effectively in poorly vascularized hypoxic tumor regions, but not in viable well-vascularized tumor regions.

Angiogenesis has also been imaged by using imaging probes that bind to integrins such as $\alpha_v\beta_3$ and $\alpha_5\beta_1$ that are expressed on the surface of endothelial cells during neovascularization [53]. Angiogenesis can be visualized *in vivo* by MRI by targeting the endothelial $\alpha_v\beta_3$ integrin that is overexpressed by tumor vascular endothelial cells [54]. The RGD peptide specifically targets the $\alpha_v\beta_3$ integrin. Recently, ultrasmall superparamagnetic iron oxide particles (USPIOs) were combined with RGD peptides to specifically target $\alpha_v\beta_3$ integrin. These RGD-USPIOs efficiently labeled the $\alpha_v\beta_3$ integrin expressing endothelial cells. The contrast generated was found to depend on the level of $\alpha_v\beta_3$ integrin expression in different xenograft models [55]. The incorporation of the antiangiogenic agent fumagillin with dual $\alpha_5\beta_1(\alpha_v\beta_3)$ -targeted paramagnetic nanoparticles to create a 'theranostic' agent was used to demonstrate the anti-angiogenic effect of fumagillin with 3D MRI in preclinical studies [53].

Imaging metastases

Since metastasis is one of the most lethal aspects of cancer, the ability to noninvasively image metastasis in preclinical models would be useful for evaluating treatments targeting the metastatic cascade. Optical imaging of PC-3 cells stably transfected to express the *FLuc* gene has been used to detect growth and dissemination *in vivo* since the optical signal is proportionate to the amount of disease burden within each animal [56]. MDA-MB-435-HAL-Luc cells have been also used successfully as a model for tracking metastases in the lungs by bioluminescence imaging [57].

Prostate-specific adenoviral vectors can function as lymphotropic agents and can mediate gene transfer into metastatic prostate cancer cells in the draining lymph nodes (Figure 2) [58]. In a recent study, orthotopically inoculated prostate cancer cells expressing *Renilla* luciferase were visualized *in vivo* by bioluminescence imaging. Mice were subsequently injected with the prostate specific adenoviral vectors AdTSTA-fl to target inguinal, iliac and lumbar lymph nodes that were identified *in vivo* by bioluminescence imaging [58].

Noninvasive optical tracking of cancer cells during metastatic progression has also been described previously [59]. MDA-MB-231 cells expressing fluorescent TdTomato-red protein were inoculated in the mammary fat pad of severe combined immunodeficient (SCID) mice and metastases in the lungs from the primary tumor were detected *in vivo* [59].

High-resolution MRI has been used to clinically detect lymph node metastases in patients with prostate cancer after injection of highly lymphotropic superparamagnetic particles (Figure 3) [60]. Following intravenous injection, these particles localize in the lymph nodes through interstitial lymphatic fluid transport. The presence of cancer cells in the lymph nodes results in an abnormal pattern of superparamagnetic particle accumulation that can be detected by MRI [60].

Image-guided drug delivery & molecular targeting

Image-guided enzyme/prodrug therapy

Damage to tissue is a major limiting factor in chemotherapy, as well as in radiation therapy, and numerous strategies that protect normal tissue while maximizing damage to cancer cells have been actively pursued. Prodrug enzyme activation systems, where enzymes delivered to the tumor convert a nontoxic prodrug to a cytotoxic drug, are one of the most attractive of these strategies but to date have resulted in limited success. Imaging the delivery of the enzyme, so that the prodrug is administered when enzyme levels are highest in the tumor and lowest in systemic circulation and normal tissues, would be of significant importance for optimizing such a therapeutic strategy. A prototype agent consisting of a cancer therapeutic prodrug enzyme labeled with multimodal MRI and optical imaging reporters was recently described (Figures 4A–C & E) [61]. The prodrug enzyme, cytosine deaminase, converts a nontoxic prodrug 5-fluorocytosine (5-FC) to 5-fluorouracil (5-FU). The prodrug 5-FC and its conversion to 5-FU can be detected noninvasively by ^{19}F MRS (Figure 4D). This prototype agent has been used in preclinical studies to demonstrate the feasibility of image-guided prodrug enzyme therapy using MRI; imaging can be used to time the administration of the prodrug when the enzyme has cleared from normal tissue but is still present in the tumor [61]. Such an approach minimizes cytotoxic side effects. This platform can be expanded to targeted delivery of the pro-drug enzyme using targeting peptides and the combination of prodrug enzyme delivery and the delivery of small-interfering RNA (siRNA). The optical reporter is especially useful to track the conjugate in cells and tissue using microscopy, while the magnetic resonance reporter provides potential clinical translatability of this approach.

Imaging drug delivery

Poor drug delivery as a result of an inefficient tumor vasculature is one of the major causes of sub-therapeutic drug concentrations within the tumor and contributes to poor therapeutic outcome. Different strategies have been described to improve drug delivery and treatment efficacy. For example, Ansiaux *et al.*, have shown that an injection of *Botulinum* toxin can potentiate cancer radiotherapy and chemotherapy in an experimental model of hepatocarcinoma [62]. The toxin induced an opening of the vascular bed, leading to an increase of tumor oxygenation and perfusion that improved tumor response to radiotherapy and to cyclophosphamide therapy. Imaging could be incorporated into designing and evaluating such strategies. Both MRI and MRSI have been used to detect drug delivery, with the potential of clinical translation.

^{13}C -labeled drugs have also been used to detect tumor drug delivery using MRSI. A recent study demonstrated the ability to image the distribution of the ^{13}C -labeled anticancer agent temozolomide (^{13}C]TMZ) in MCF-7 tumor-bearing mice using $^1\text{H}/^{13}\text{C}$ MRSI. Detection of ^{13}C]TMZ was achieved in the micromolar range. 3D maps of drug distribution with a nominal 2.5-mm isotropic resolution were obtained following intraperitoneal administration of 200 mg/kg ^{13}C]TMZ [63].

Efforts are also being made to design novel carriers for image-guided drug delivery, such as magnetic nanoparticles, consisting of a magnetic core and a biocompatible polymeric shell (Figure 5) [64]. In addition to being detected with MRI, the local application of an external magnetic field improved the accumulation and retention of these particles in mouse tumors following systemic injection [64].

Liposomes are useful as carriers to deliver drugs and molecular targeting agents to tumors. One advantage of liposomes is that they can be noninvasively imaged to ascertain their delivery within the tumor. Liposomes labeled with magnetic resonance contrast agents or fluorescent

probes have been imaged *in vivo* and *ex vivo* [65]. Liposomes can be targeted, for instance, to the tumor vasculature by coating their surface with specific antibodies or peptides. By adding biotin to RGD-functionalized liposomes, avidin chase can be used to increase the specificity of targeting tumor angiogenesis *in vivo* [33].

Molecular targeted therapy

The search for highly effective cancer treatments with minimal or no side effects continues to be a major challenge for our decade. The discovery of RNA interference (RNAi) mediated by siRNA has provided powerful new tools for biological research and drug discovery and siRNA silencing holds great promise in cancer therapy to knock down overexpressed genes involved in cancer progression and metastasis. The delivery of siRNA *in vivo* is, however, a major challenge, owing to rapid elimination of siRNA by the kidney, fast degradation by serum RNases and the low intracellular permeability of naked siRNAs. There is, therefore, an urgent need for the development of siRNA delivery vectors that introduce siRNAs efficiently and specifically into the cytoplasm and that can be imaged. Among the siRNA delivery vectors that have been studied, viral vectors usually provide the highest transfection efficiencies. However, safety concerns arising from potential mutations, recombination, oncogenic effect, high cost and issues related to immune response limit their clinical translation.

In a recent preclinical study, lentiviral vector-mediated downregulation of choline kinase using short hairpin RNA (shRNA), was demonstrated in a human breast cancer xenograft [66]. Increased phosphocholine and total choline are usually observed in most cancer cells and silencing of choline kinase (the enzyme that converts choline to phosphocholine) has been found to reduce cell proliferation and induce differentiation [67]. Concentrated lentivirus, expressing shRNA against choline kinase, was injected intravenously in the tail vein of MDA-MB-231 tumor bearing female SCID mice. Transduction efficiency in cells and tumors *in vivo* was determined optically by enhanced GFP expression. Noninvasive ³¹P magnetic resonance spectra of choline kinase–shRNA-transduced tumors *in vivo* showed lower phosphocholine and phosphomonoester levels that were associated with reduced tumor growth and proliferation. The study demonstrated the use of lentiviral vectors to target choline kinase in a human breast cancer xenograft and the use of noninvasive MRS to detect this targeting.

Compared with viral vectors, nonviral delivery vectors provide an attractive alternative owing to advantages such as easy and controllable synthesis, low immune response and safety. Among the nonviral siRNA delivery vectors available, cationic liposomes are currently one of the major systems used for systemic delivery of siRNA. The complexation between the negatively charged siRNA and the positively charged nonviral vectors is mainly driven by electrostatic interactions. The resulting complex enhances siRNA uptake by:

- Increasing the circulation lifetime and biocompatibility of siRNA;
- Protecting the integrity of siRNA in the blood;
- Changing the siRNA-associated charge density and polarity, which enhance the interaction with the negatively charged cellular membrane.

Delivery of COX-2-specific siRNA using liposome tagged with fluorescent and magnetic resonance probes has recently been demonstrated [65]. siRNA conjugated to a dual-purpose probe containing magnetic nanoparticles for MRI, and Cy5.5. for NIRF has been used to efficiently target the survivin gene, inducing an increase in apoptosis and necrosis of the tumor cells *in vivo* [68].

Quantitative *in vivo* MRSI has also been used to determine the regional activity and concentration of an exogenous enzyme expressed from a therapeutic transgene [69]. In this study, mice were inoculated with cancer cells expressing cytosine deaminase-uracil

phosphoribosyl-transferase and ^{19}F MRSI was performed to assess the kinetics of 5-FU in the tumors [69]. The rapid anabolism of 5-FU by UPRT resulted in a high level of 5-FNuc that was observed in ^{19}F spectra (Figure 6). Interestingly, 5-FNuc-induced apoptosis was observed in tumor regions exhibiting UPRT activity and expression.

Detecting treatment

Functional imaging, a traditional strength of MRI and MRS techniques, can be usefully integrated to detect the effects of anti-angiogenic or antivascular treatment and to understand the functional effects of therapeutic agents such as radiation and chemotherapy [70,71]. USPIO-enhanced MRI was used to detect the effect of thrombogenic vascular-targeting agents [72]. Quantitative maps of tumor perfusion could identify responding from non-responding mice in a fibrosarcoma tumor model [72].

A Gd^{3+} -based targeted MRI contrast agent that can be used to detect tumor cell death *in vivo* by binding to exposed phosphatidyl-serine was recently described [73]. This *in vivo* binding was detected by an increase of signal intensity in T_1 -weighted images, due to the shortening of water T_1 by Gd^{3+} . Such contrast agents can assess early efficacy of cytotoxic treatment by detecting dying cells before changes in tumor size.

The use of hyperpolarized ^{13}C allows an improvement in the sensitivity of detection of the magnetic resonance signal of ^{13}C -labeled substrates of the order of 10,000 [74]. This increase in the sensitivity of detection of ^{13}C -labeled substrates is being applied to detect treatment response, as shown by Gallagher *et al.* [75]. In the study, the formation of $[1,4\text{-}^{13}\text{C}_2]\text{malate}$ was detected *in vivo* in a murine lymphoma tumor, following the injection of hyperpolarized $[1,4\text{-}^{13}\text{C}_2]\text{fumarate}$. Results from this study suggest that the formation of $[1,4\text{-}^{13}\text{C}_2]\text{malate}$ may be used to identify the formation of necrosis following treatment [75].

By combining both vascular MRI and ^{19}F MRS, Baker *et al.* demonstrated that irinophore C treatment improved the vascular function of tumors and increased the delivery and accumulation of 5-FU [76].

Diffusion-weighted imaging (DWI) and dynamic contrast-enhanced (DCE) MRI have been combined to develop new suitable biomarkers for early detection of anti-angiogenic treatment efficacy in a bone metastasis model [77]. Experimental bone metastasis was induced in rats by an intravenous injection of MDA-MB-231 breast cancer cells. Bevacizumab, an anti-VEGF antibody, was used as an anti-angiogenic treatment. DCE-MRI detected an early response characterized by a decrease in the amplitude signal that was suggestive of decreased new vessel formation and regression of immature vessels. This was confirmed by histology [77]. However, although DWI is sensitive for detecting the effects of cytotoxic treatments [56], it failed to detect early response to the anti-angiogenic treatment despite a decrease in tumor volume [77].

Medarova *et al.* recently developed a new multimodal imaging probe that binds to the underglycosylated mucin-1 tumor antigen, overexpressed in breast tumors [78]. This probe contained a superparamagnetic iron oxide particle, which was detected with MRI and a Cy5.5 dye, detected with NIRF optical imaging and peptides that specifically recognized underglycosylated mucin-1 tumor antigen. MRI and NIRF imaging were combined to show the efficacy of doxorubicin treatment in a breast cancer model [78]. The probe provided molecular information about target antigen expression and represents a new semiquantitative approach to assess therapy-induced changes by *in vivo* MRI [78]. A combination of MRI and FMT has been used to evaluate morphological and functional changes in tumors in response to chemotherapy by monitoring tumor growth and protease activity [45]. Protease activity was

quantified by injecting ProSense, a chemical probe that exhibits specific NIRF after cleavage by lysosomal cysteine and serine proteases [45].

Future perspective

Finding effective treatments against cancer depends upon identifying and attacking targets and pathways critically important for the cancer cell, with the additional caveat that each cancer represents an individual disease and that no two cancers may be alike. As such critical targets in cancer cells are revealed, this decade is witnessing a transition in cancer treatment from the 'sledgehammer' approach of conventional chemo- and radiation-therapy to specific molecular targeting. The success of these molecular-targeted treatments depends upon the availability of noninvasive imaging techniques to select targeted therapies that would be most effective against a particular cancer and to detect response [43]. One of the major promises of molecular functional imaging in cancer is therefore to achieve 'personalized treatment'. With its current trajectory, the integration of imaging with chemistry should result in the design of novel probes and contrast agents that might minimize the use of invasive biopsy procedures in characterizing cancers and predicting which therapeutic option would be ideal for this personalized treatment.

The identification of specific targets in cancer is also driving advances in novel image-guided platforms such as nanoparticles [68], liposomes [65] and microencapsulation devices [79], to deliver siRNA to downregulate these targets and pathways.

One of the most understandably sought-after goals in cancer management is the development of noninvasive biomarkers to predict risk, allow early identification, assist in the selection of treatment and detect response [80]. The bench-to-bedside span of noninvasive magnetic resonance methods to characterize cancer makes them an attractive choice in the identification and development of noninvasive biomarkers. Through the development of novel molecular-targeted imaging probes, the traditional functional imaging strengths of MRI and MRS have expanded to include visualization of cell surface receptors [31], molecular pathways, degradative enzyme activity [42] and the ECM integrity [48] in preclinical models. These novel targeted bio-markers can be used to follow the response of tumors to molecular-targeted treatments.

One major direction for the future will be to translate preclinical developments for use in the clinic. Unlike radiopharmaceuticals, the inherent insensitivity of magnetic resonance methods will require that most molecular magnetic resonance contrast agents be used at concentrations that will be subject to much more stringent control by the US FDA.

Administering gadolinium-based contrast agents to patients with kidney disease is becoming a concern given the risk of nephrogenic systemic fibrosis [81]. Although additional investigations are required before drawing definitive conclusions [82], these agents can no longer be assumed to be without serious side effects [83]. Recent studies have focused on finding alternative magnetic resonance contrast agents, such as USPIO particles, for patients at risk of nephrogenic systemic fibrosis [81]. Challenges for the future will be to increase the sensitivity of detection of such agents through novel chemistry or signal-amplification strategies such as hyper-polarization [74,84] or instrumentation, and the incorporation of multimodality imaging.

Two key problems facing clinical cancer imaging, especially brain cancer, are the identification of true tumor margins and distinguishing treatment changes versus recurrence. Developing probes that can assist in accurate delineations of tumor margins, especially in invasive cancers, would benefit radiation treatment planning and surgery. Similarly, the ability to differentiate changes occurring due to treatment, from changes due to the tumor recurrence would be valuable in guiding therapy decisions and options.

Despite the tremendous strides in cancer research and treatment that have been made in the 21st Century, cancer still evades cure and frequently control. Tumor recurrence and metastasis continue to be the leading causes of morbidity and mortality from cancer. The discovery of cancer-initiating cells or 'cancer stem cells' is generating tremendous excitement and is offering new paradigms for understanding and treating cancer recurrence and metastasis. These stem-like cancer cell populations can form tumors in immune-suppressed mice from low cell number inoculums, are more drug resistant, more invasive and more likely to metastasize [85,86]. Identifying or developing probe-based biomarkers associated with cancer stem cells and imaging and targeting permissive or preventive micro-environmental niches for cancer stem cells is another area that will have significant impact on cancer research and treatment.

Executive summary

- Cancer is a multifaceted disease with metastasis/recurrent disease continuing to be a major cause of mortality. The multifaceted nature of cancer requires individualized characterization and treatment. A continuing challenge in cancer is to selectively eliminate cancer cells, either through destruction or differentiation, without damaging normal tissue.
- Chemistry is the new frontier in advancing applications of molecular functional imaging in cancer. Designing and developing novel imaging probes that can be integrated with imaging to detect and comprehensively characterize the tumor and identify the best treatment option will advance the concept of 'personalized treatment' for cancer. It will also lead to the discovery of novel targets for treatment.
- Both MRI and optical imaging techniques are useful in preclinical models of cancer. Both techniques have advantages and limitations. Although MR methods are translational, unlike the nanomolar detection limits of positron emission tomography and single-photon emission tomography agents that result in minimal toxicity, the poor sensitivity of MR and the associated requirement of using contrast agents at high concentrations, necessitates new amplification strategies or the combination of MR with nuclear imaging techniques.
- Some of the broad areas that the integration of chemistry with imaging can impact upon in cancer discovery and treatment include uncovering and exploiting new targets to exploit for cancer treatment, detecting delivery of therapeutic agents and exploiting this ability to minimize normal tissue toxicity, identifying which tumors are most likely to respond to a specific treatment and detecting this response by designing novel imaging probes as biomarkers.

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Key Terms

Angiogenesis	Formation of new blood vessels from existing vessels that is induced by angiogenic factors secreted by cancer cells and the stromal cells that the tumor co-opts
Invasion	Process by which cancer cells degrade and invade into the extracellular matrix

Metastasis	Process by which cancer cells intravasate into lymphatic and blood vessels, circulate through the bloodstream and extravasate at distant sites to establish metastatic colonies
MRI	Noninvasive method that detects radiofrequency signal from nuclear spins, which are precessing in a static magnetic field and have been excited with a radiofrequency pulse
Optical imaging	Primarily used for gene expression <i>in vivo</i> and requires that gene expression be coupled to the emission of light photons that can travel through tissue. The light photons should lie within the spectral window of low tissue autofluorescence, photon attenuation and light scattering. Molecules such as water and oxygenated and deoxygenated hemoglobin, which are highly abundant in biological tissues, absorb light in the lower portion of the visible spectrum, as well as in the infrared region of the spectrum
Imaging probes	Used in combination with imaging modalities to detect and characterize specific molecular and functional parameters in tissues <i>in vivo</i> usually after injecting the probe

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Figure 1.

In vivo white light and fluorescent imaging of mice bearing PC-3 tumors in the left flank and LNCaP in the right flank following injection of hepsin specific binding peptides conjugated to a fluorescent nanoparticle.

Adapted with permission from [37].

Figure 2. Detection of lymph node metastasis in an orthotopic xenograft model of prostate cancer (A) Immunohistochemistry of LAPC-9-VEGF-C-RL orthotopic tumor showing extensive lymphatic network (red) along the margin (dotted white line) of the tumor (Tu.) extending intratumorally from the normal prostatic region (Pros.). (B) Representative optical image of *Renilla* luciferase expression (left) at 3 weeks after orthotopic implantation. Firefly luciferase signal is observed (right panel) in inguinal (left, IL; right, IR) and periaortic (PA) regions after injection of AdTSTA-fl in both hind paws of mice carrying LAPC-9-VEGF-C-RL orthotopic tumors. (C) *Ex vivo* firefly luciferase bioluminescence activity of dissected nodes. Inguinal node (Ing.). (D) Histological analysis (H&E) showed the presence of a subcapsular lesion within the periaortic lymph node. This mass was verified to be LAPC9 tumor tissue by staining with anti-CK in an adjacent section. Scale bars: (A) 200 μm ; (D) 100 μm . Anti-CK: Antibody to human cytokeratin; H&E: Histological analysis; PA: Periaortic; Pros: Prostatic region; Tu: Tumor. Adapted with permission from [58].

Figure 3. Imaging lymph node metastasis in a prostate cancer patient

(A) 3D reconstruction of the prostate, iliac vessels and metastatic (red) and nonmetastatic (green) lymph nodes. A malignant node (thick arrow) is observed immediately adjacent to a normal node (thin arrow). (B) Conventional MRI showing increased signal intensity identical in the two nodes. (C) MRI with lymphotropic superparamagnetic nanoparticles showing decreased signal intensity in a normal node (thick arrow) but high signal intensity in a metastatic node (thin arrow).

Adapted with permission from [60].

Figure 4. Image-guided prodrug enzyme therapy

(A) High resolution T_1 -weighted MR image and (B) T_1 map of MDA-MB-231 breast cancer xenograft before and after intravenous injection of bCD-PLL. Delivery of bCD-PLL is evident from the enhanced signal in the T_1 -weighted image that corresponds to lower T_1 values in the T_1 map. (C) *In vivo* optical image of mouse bearing MDA-MB-231 xenograft pre- and post-injection of bCD-PLL. (D) *In vivo* time-dependent ^{19}F MRS of an MDA-MB-231 breast cancer xenograft with 5-Fc (450 mg/kg i.v.) injected 2 h after administration of bCD-PLL (1.00 g/kg i.v.). MRS acquisitions (26 min/acquisition) started 1 h after the prodrug injection. (E) *In vivo* time dependent T_1 value and average radiance (inset) of the MDA-MB-231 xenograft after intravenous injection of bCD-PLL.

i.v.: Intravenous; MRS: Magnetic resonance spectroscopy.

Adapted with permission from [61].

Figure 5. Monitoring of nanoparticle accumulation by MRI with (A) targeted and (B) nontargeted agents

The maps are representative series of R_2 maps (ms^{-1}) of the tumor tissue (in color) superimposed with the corresponding T_2 -weighted images acquired before (baseline), 1 h and 3 h after the nanoparticle injection.

Adapted with permission from [64].

Figure 6. *In vivo* imaging of transgene activity

^{19}F -magnetic resonance spectroscopy image and associated parametric map demonstrating circumscribed regions of cytosine deaminase-uracil phosphoribosyltransferase (CD-UPRT) levels and activity within a CD-UPRT⁺/CD-UPRT⁻ cotransplanted tumor xenograft. Adapted with permission from [69].

Table 1

Advantages and limitations of MRI and optical imaging techniques.

Modality	Advantages	Limitations
Fluorescence and bioluminescence imaging	High sensitivity in nanomolar range (>50 cells [fluorescence]; ≥ 1000 cells [bioluminescence]) High specificity Quantitative and longitudinal assessment Cost effective Noninvasive	Predominantly preclinical, limited clinical translation Restricted depth detection due to attenuation of the signal Low spatial resolution <i>in vivo</i> Limited access to tissue anatomy
FMT	3D reconstruction and quantification possible Imaging tissues at depths of <10 cm	Predominantly preclinical, limited clinical translation Low spatial resolution <i>in vivo</i>
SHG	No labeling Specificity of the signal High spatial resolution Cost effective	Limited depth detection ability (<500 μm) Currently, limited <i>in vivo</i> application Only detects optically noncentrosymmetric structures
OCT	Imaging of near surface abnormalities No labeling Microscopic information of tissue anatomy High spatial resolution ($\sim 10 \mu\text{m}$) Cost effective	Limited depth detection ability (<1 cm) Currently, limited <i>in vivo</i> application
MRI	Preclinical and clinical applications Access to tissue anatomy and physiological functions High spatial resolution (10–100 μm) and soft tissue contrast Possibility of quantification No depth limit and noninvasive	Low sensitivity to detect contrast agents Cost Acquisition time Particle size of contrast agent often large may restrict <i>in vivo</i> delivery
MRSI	Preclinical and clinical applications Access to physiological functions and metabolism Possibility of quantification No depth limit Noninvasive	Low sensitivity Limited spatial resolution Cost Acquisition time

FMT: Fluorescent magnetic tomography; MRSI: Magnetic resonance spectroscopy imaging; OCT: Optical coherence tomography; SHG: Second harmonic generated.