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Editorial

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Role of Molecular Imaging in Oncology

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ABSTRACT

Molecular Imaging (MI) is an emerging technology for the early detection of disease, staging of the disease, and for monitoring response to therapy. It also offers a non-invasive method to detect *in vivo* biological functions and processes at a molecular level. The use of MI requires careful selection of targeting molecules which are expressed differentially in diseased *vs.* healthy cells to interrogate the cell microenvironment. Targeting molecules for MI could consist of small molecules, single amino acid units, low molecular weight peptides, antibodies or antibody fragments. Over the years, a large number of small molecular weight imaging probes have been developed to target different molecular pathways using Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT). The use of high molecular weight probes such as radioactive antibodies (Ab) is also equally attractive. While a major effort is placed on developing radioactive probes for PET and SPECT imaging, an intense effort is being focused on enhancing the utility of other MI modalities such as nuclear Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS), Computed Tomography (CT), optical imaging and ultrasound (US).

KEYWORDS: Antibody; Imaging; SPECT; PET; Tumor; Optical.

INTRODUCTION

Molecular Imaging (MI) is an emerging field that combines the non-invasive monitoring of *in vivo* biological processes at the cellular level and the anatomic information associated with tissues undergoing such transformations. MI therefore has evolved as an indispensable technique for the early detection and diseasestate, and for monitoring response to therapy. These modalities offer non-invasive detection of *in vivo* biological functions and processes at the molecular level through the careful selection of targeting molecules which is expressed differentially in diseased *vs.* healthy cells to interrogate the cell microenvironment. These targeting molecules could consist of small molecules, single amino acid units, low molecular weight peptides, antibodies or antibody fragments. There are now a large array of imaging technologies under the MI umbrella which include Single Photon Emission Computed Tomography (SPECT) and Positron Emission Tomography (PET), Magnetic Resonance Imaging (MRI), Magnetic Resonance Spectroscopy (MRS), Computed Tomography (CT), ultrasound (US), bioluminescence (quantum dots), and fluorescence imaging (optical imaging).[1] Amongst the tools required for targeting diseased tissues to obtain clinically relevant information, the key is to match the optimal targeting molecule with the imaging modality of choice.

RESULTS AND DISCUSSIONS

Amongst various options within MI, nuclear imaging is the more common non-invasive detection technique to target tumors. Nuclear imaging employs radiolabeled tracers which concentrate in cancerous tissues and generates the radioactive signal for visualization of tu-

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-mors. This signal is detected, reconstructed, and analyzed with the resulting image corresponding to the spatial distribution of the radiotracer in the cancer mass and surrounding tissues. The two major nuclear imaging modalities are SPECT and PET.

POSITRON EMISSION TOMOGRAPHY/COMPUTERIZED TOMOGRAPHY (PET/CT)

PET, a non-invasive imaging modality, allows quantitative evaluation of the biological processes *in vivo* through emission of positrons from nuclear decay of an intravenously injected radiopharmaceutical. PET typically has higher sensitivity than conventional SPECT which allows the targeting of sites at much lower concentrations of radiotracers in the target area, allowing localization of small lesions.

One of the most commonly used radiotracers in MI is F-18 fluorodeoxyglucose (FDG), a compound approved by the FDA for clinical use. Since membrane glucose transporter (GLUTs) expression increases significantly in rapidly dividing cancer cells, FDG enters these cells producing preferential uptake and phosphorylation of FDG and permitting clinically meaningful imaging of the tumor. Besides FDG, there have been many advances in developing new class of radiotracers to diagnose cancer and to interrogate certain neurological processes. O-15 water (blood flow),[2] F-18 labeled fatty acids (fatty acid/thiokinase metabolism),[3] F-18 FLT (thymidine kinase),[4] F-18 fluoroestradiol (estrogen receptor targeting),[5] C-11 and F-18 choline (choline kinase/oncological imaging),[6-11] F-18 fluoromisonidazole (F-MISO; hypoxia imaging),[12,13] radiolabeled annexin (apoptosis),[14,15] F-18 FMDHT (androgen receptor imaging),[16,17] and F-18 FHBG and analogues (reporter gene targeting/imaging),[18-20] are some of the examples illustrating a wide scope and contribution of this imaging modality to molecular imaging field. Similarly, SPECT uses radionuclides which decay through a single photon emission branch. As with PET, SPECT also requires the use of radioactive probe for MI, albeit the chemistry involved in preparing those radioactive molecules could be quite different.

While the choice of radiotracer varies with application, several of these targeting probes are multifunctional and have a widespread utility across many diseases. Most of these probes are small molecules, have rapid clearance from blood pool (circulation), rapid washout from normal tissues and low metabolic disintegration. Our own and other researchers experience with C-11 choline in patients with prostate cancer has been quite impressive.[7,21-23] Patients with recently diagnosed prostate cancer were scanned using C-11 choline to localize the tumor. At the conclusion of the treatment regimen, the patients returned for a second C-11 choline scan to assess treatment efficacy. As shown in figure 1, PET/CT imaging with C-11 choline has been quite unequivocal in monitoring therapy outcome.[6]

Similarly, C-11 choline plays an impressive role in following treatment response in patients with esophageal cancer.[24] Several other PET imaging probes labeled with F-18 have also been developed over the years.[6,10,16,17,25,26,27] Whole body PET/CT imaging with 7α-[^{18}F] fluoro 17α-methyl 5α-dihydrotestosterone (F-18 FMDHT) in normal healthy volunteers is shown in figure 2. The radiotracer clears from most normal tissues following the hepatobiliary clearance. The initial uptake in the urinary bladder was low but increased after 60 min post injection, indicating its potential to clearly discern primary as well as metastatic prostate cancer from normal tissues.

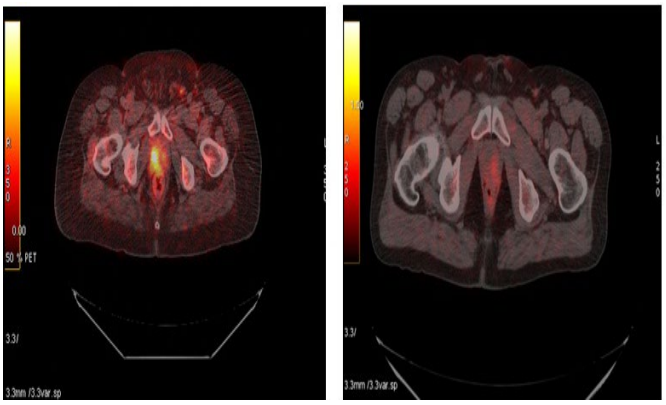

Figure 1: PET/CT image of a patient with prostate cancer before and after therapy. The left panel shows C-11 choline uptake in the prostate bed (scan acquired prior to therapy). After this scan patient underwent therapy. The right panel shows the PET/CT scan after patient completed therapy. No uptake of C-11 choline is noted in that region supporting the efficacy of C-11 choline to monitor prostate cancer therapy.

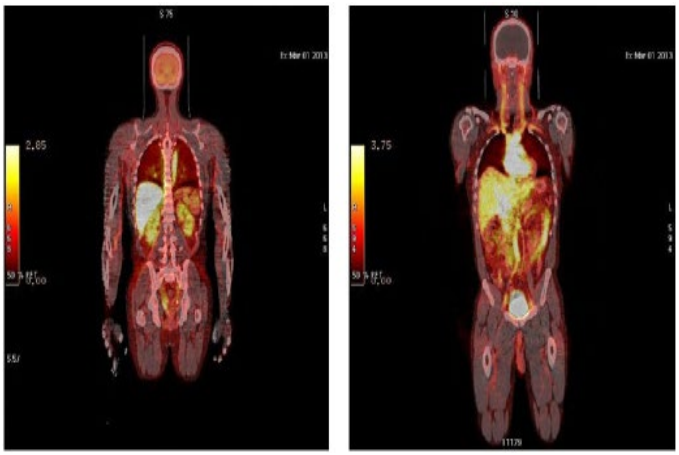

Figure 2: Distribution of F-18 FMDHT in normal healthy volunteer. Early image (left panel) show uptake of F-18 in the liver, spleen, kidneys. Late image of same patient (2 h post injection) show significant clearance of radioactivity from liver through the hepatobiliary path with radioactivity accumulation in the bladder.

While small molecular weight compounds have already been proven effective for molecular imaging applications, the role of large proteins such as antibodies has been steadily gaining momentum. One of the key parameters is to non-invasively visualize target molecules in altered cells by virtue of the target probe interaction at the molecular level. While some evidence suggests the clinical utility of Ab in molecular imaging, because of their large molecular size (150 kDa), the intact immunoglobulins remain in circulation[28] and may take a long time to accumulate in tumors (1-2 days) This slow uptake

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decreases the clinical utility of such a probe and investigators have been less than enthusiastic to consider such molecules as relevant for molecular imaging. Advancement in antibody engineering leading to small molecular weight constructs such as monovalent fragments (variable fragments Fv, single chain variable fragments (scFv), bivalent or bispecific diabodies, tribodies, Fab fragments and minibodies) raised the enthusiasm in the MI community to explore their utility in imaging. In comparison to the intact antibody, these smaller fragments, particularly the single chain antibodies $(scFv)_2$ show faster clearance from the blood and rapid peak tumor localization. Over the years, a number of antibody based tumor biomarkers have been developed for the diagnosis or treatment follow-up of specific cancers.[29-31] However, challenges to the effective exploitation of differentially expressed markers for the timely visualization of growing tumors remain. With the recent initial successes in preclinical and translational studies, several such antibody based entities are now FDA approved for clinic use. Some of the examples of antibody based radiopharmaceuticals are OncoScint CR/OV (Satumomab Pendetide) which targets the cell surface mucin-like glycoprotein antigen TAG-72 in colorectal and ovarian carcinomas, ^{111}In-labeled Oncoscint for pre-operative evaluation, monitoring recurrence,and detection of extrahepatic metastasis,[32] and ProstaScint (CapromabPendetide) for the diagnosis of metastatic prostate cancer.[33,34] A radionuclide with a longer half-life such as ^{124}I (4.18 d) has helped to further explore the use of whole IgG because the long half-life radionuclide allows for extended time for uptake in targeted sites while allowing for the clearance from normal tissues and from non-specifically circulating fraction[35] and maintaining enough radioactivity to produce a useful imaging signal. More recently, Zirconium-89 (^{89}Zr), a transition metal, has turned into an attractive choice of radionuclide for PET imaging due to its longer half-life (3.3 days) which is compatible with the pharmacokinetics of large molecules such as antibodies and their fragments. ^{89}Zr-labeled mAb has been proven to be stable in vivo and has provided better spatial resolution than that with ^{18}F FDG PET. Use of ^{89}Zr labeled mAbs as a scouting procedure preceding radioimmunotherapy has been proposed.[36-38] In one of the earliest studies, ^{89}Zr was labeled with mAb U36 recognizing the v6 domain of CD44, a tumor antigen overexpressed in head and neck tumors. This radioimmunoconjugate was used in preclinical models to confirm tumor targeting and estimate the dose deposition to tumor and normal tissues prior to radioimmunotherapy with ^{90}Y-labeled mAbs.[39]

Another radionuclide with attractive PET properties and compatible with large as well small molecular weight targeting molecules is copper-64 (^{64}Cu). Initial efforts using copper-64 explored the use of macrocyclic chelating agent 1,4,7,10-tetraazacyclododecane-N,N',N'', N'''-tetraacetic acid (DOTA). This radiolabeled small molecular weight antibody (minibody; 80 kDa) cleared rapidly from circulation and exhibited moderate tumor uptake shown by serial imaging of antigen positive tumors using preclinical models and micro PET imaging.

OPTICAL IMAGING

The use of optical imaging is also rapidly being adopted by molecular imaging scientists. Optical imaging includes fluorescence and bioluminescence based imaging probes. Optical imaging modalities are very promising due to their high sensitivity and specificity, low cost, portability of imaging instruments and absence of ionizing radiation. Further with the imaging is showing potential for clinical relevance. In fluorescence imaging, cells are labeled with dyes or proteins which emit light of a limited spectrum when excited. Bioluminescence methods use an enzymatic reaction between a luciferase enzyme and its substrate, luciferin, to produce photons which are converted to electrons and detected by a cooled Charged Couple Detector (CCD). This ultimately results in the detection of visible light through near-infrared light signals. Fluorescent molecules like Green Fluorescent Protein (GFP) and Red Fluorescent Protein (RFP) are used to observe the localization of proteins within a cell, to label specific proteins, or to monitor the production of a gene product.[40] For bioluminescence imaging, the bioluminescence reporter Firefly Luciferase (FL) and Renilla Luciferase (RL) are used frequently.

More recently, advances have been made in the field of infrared imaging. Near-Infrared imaging (NIR) has deeper penetration ability and higher sensitivity than the previously discussed optical imaging options. Ogawa et al., analyzed the efficiency of NIR flourophorindocyanine green (ICG) for *in vivo* imaging and produced encouraging results.[41]

ULTRASOUND IMAGING (US)

Ultrasound (US) is a cross-sectional imaging modality which uses sound-waves to produce interpretable images. Pulses of sound-waves of appropriate frequencies (usually 1-20 MHz depending on desired imaging depth) are emitted through arrays of transducers (probes).The sound-waves are reflected at tissue boundaries and these returning echoes are captured and reconstructed to produce a two-dimensional image of the plane being scanned. The resulting image is displayed using a grey-scale display corresponding to the intensity of the returning echoes. Ultrasound contrast agents are a relatively new concept and are designed to alter the absorption, reflection or refraction of the sound-waves which enhance the differentiation of the signal from the tissue containing the contrast agent from that of the surrounding sample. Targeted ultrasound contrast agents are site-directed contrast agents which specifically enhance the signal from pathologic tissue which would otherwise be difficult to distinguish from surrounding normal tissue. Microbubbles (MB) are US contrast agents which are gas filled structures encapsulated by lipid shell, polymer or proteins and when injected into the blood stream enhance signals from the target tissues.[42,43] Their low cost, easy transportability and utility to provide a discriminated signal from

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background with high sensitivity has made the MB contrast agent of choice for US imaging. Future research could allow specific ligands to be attached to the MB's surface which could lead to the accumulation of these agents in the target sites. Because of their large size, MB rarely extravagates from vascular tissue. Also, the MB targeted to the platelet glycoprotein IIb/IIIa integrin[44] and to fibrin[45] have been used for thrombus imaging. Fortunately, targeted MB based contrast imaging is even making functional characterization of tumor vasculature possible.

Although, there are manuscripts reporting that only a small number of MBs are required to produce adequate contrast for imaging purposes, further research is needed to assess the overall sensitivity of targeted microbbubles, determine the amount of antibody sufficient to get the target microbubble to the desired anatomic location, and to determine the comparative efficacy of MBs in comparison to nuclear and other optical imaging modalities.

MAGNETIC RESONANCE IMAGING (MRI) AND MAGNETIC RESONANCE SPECTROSCOPY (MRS)

MRI primarily uses the magnetic resonance signal produced from the hydrogen atoms in a sample under the influence of strong magnetic field for constructing three dimensional image sets. Positive and negative contrast agents containing metal ions are used to enhance MRI sensitivity. Positive contrast agents (appearing bright on MRI) are small molecular weight compounds usually containing gadolinium (Gd), manganese (Mn) or iron (Fe) as their active element. Negative contrast agents (appearing predominantly dark on MRI) are small particulate aggregates such as Super Paramagnetic Iron Oxide (SPIO) or Ultra-small Super Paramagnetic Iron Oxide (USPIO) particles which have an iron oxide core containing iron atoms covered by a polymer shell usually made of dextran or polyethyleneglycol. Although contrast enhanced MRI is a powerful imaging modality with sub-micrometer resolution (10-100 µm) and exquisite soft tissue contrast, its major drawbacks are low sensitivity, low retention of positive contrast agents, relatively long imaging times, and the necessity to inject large amounts of contrast to obtain quality images.

Since conventionally used contrast agents like Gd lack specificity, targeted contrast agents are being developed by conjugating paramagnetic compounds to various peptides, ligands, antibodies and antibody fragments that target specific tumor cell surface antigens. Superparamagnetic Iron Oxide (SPIO) is another class of negative contrast agents used in MR imaging.

Nanoparticles are nano-sized carriers used for delivery of various pharmaceuticals. In recent years, there has been intense effort to develop multifunctional nano-carriers for the targeted delivery of diagnostic and therapeutic agents. These multifunctional nano-carriers could incorporate antibodies for specific and effective tumor targeting and delivery of therapeutic payloads. For example, nano-shells are optically tunable nanoparticles that contain a dielectric core surrounded by a thin gold shell. These nano-shells may be designed to scatter and/or absorb light over a broad spectral range. Thus depending on wavelength of light chosen, these nanoparticles can be utilized for imaging and for photo thermal therapy.

OTHER IMAGING MODALITIES

Raman spectroscopy is based on the inelastic scattering of light during its interaction with matter. Although the light scattered from biological samples is usually weak, the intensity of scattered light increases tremendously in the vicinity of metallic nanoparticles. These metallic nanoparticles, on excitation at a particular wavelength, exhibit surface plasmon resonance and resonate light. This resonation results in enhanced scattering from these metallic particles which result in increased contrast when compared to the surrounding molecules. The increased contrast is basis for Surface Enhanced Raman Spectroscopy (SERS). Due to its ability to detect picomolar amounts of the targeted molecule, SERS is becoming a predominant imaging modality. SERS provide multiple advantages over existing imaging modalities including the unique spectral properties of SERS nanoparticles with easily resolved narrow peak which allows for multiplex imaging ,lower toxicity due to inert nature of SERS nanoparticles (Gold, silica). Moreover, colloidal gold particles coated with a protective layer of polyethylene glycol (PEG) exhibit excellent *in vivo* biodistribution and pharmacokinetic properties upon systemic injection, and SERS probes provide enhancement of the Raman scattering of adjoining molecules by as much as 10^{14}-10^{15} which can lead to the detection of a single molecule.[46,47] SERS is becoming the modality of choice when localized and surface tumors. However, its use remains limited in imaging deep tumors due to shallow depth of penetration associated with Raman microscopes and the non-specific uptake of these particles.[48]

CONCLUSION

A wide array of imaging modalities is available to the preclinical research community and many of these modalities have been adopted by the clinic. Nonetheless, the efforts to further optimize probes to more precisely target cellular level processes with high specificity and selectivity continues to be an area of much research effort. As we gain more knowledge on the molecular interactions of various disease states, new and novel probes are being considered, explored, and designed. Along with probe development, or perhaps because of it, there has been great progress in enhancing the capabilities of imaging instruments and techniques to further help visualize these molecular processes with increased sensitivity and resolution. As the molecular imaging field is evolving and scientists and physicians are recognizing its importance, industries are tak-

-ing note of the strength of this modality. Several pharmaceutical companies have either entered in collaboration with researchers from various academic institutions or have developed an in-house molecular imaging program. It is only a matter of time when molecular imaging will become an integral part of drug-development to provide routine standard of care in the clinic.

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