Molecular Imaging Applications

Zaver M. Bhujwalla

JHU ICMIC Program, Division of Cancer Imaging Research, The Russell H. Morgan Department of Radiology and Radiological Science, The Johns Hopkins University School of Medicine, Baltimore, MD 21205. E-mail: zaver@mri.jhu.edu

Highlights
Overview of molecular imaging
MR molecular imaging within the context of other imaging modalities
MR molecular imaging strategies

Target Audience
M.D. and Ph.D. researchers with an interest in molecular imaging concepts and applications.

Introduction
Molecular imaging has revolutionized our perceptions of imaging. This high impact field is finding transformative applications in the understanding, detection, and treatment of nearly all diseases [1].

The field of molecular imaging is an exciting fusion and integration of many different disciplines including molecular biology, chemistry and probe design, imaging technologies, visualization, and image analysis, that are focused on understanding, detecting, and treating oncological, neurological, cardiovascular, inflammatory, metabolic, and infectious diseases (Figure 1). Based on their strengths, different imaging modalities provide different but equally valuable information that can be integrated in advancing our understanding of these diseases.
As the era of ‘omics’ progresses towards personalized medicine, the field of molecular imaging is finding multiple uses in noninvasive characterization of the molecular features of diseases and their impact on function. In complex diseases such as cancer, with its tremendous genetic diversity, it is becoming increasingly important to develop molecularly-targeted treatment strategies that combine detection with treatment.

MR molecular imaging within the context of other modalities
A summary of different imaging modalities and their sensitivity, spatial resolution, strengths and weaknesses are presented in Table 1 [2] and a summary of the different applications of MR techniques are summarized in Table 2. These tables highlight the advantages of MR and its strengths in imaging metabolism and physiology. However, a drawback of MR methods is limited sensitivity of detection and several innovative strategies are being pursued to increase these limits.

### Table 1: Sensitivity, spatial resolution, and clinical translation of molecular-functional imaging (MFI) modalities [2].

<table>
<thead>
<tr>
<th>Imaging Modality</th>
<th>Sensitivity of detection in MFI</th>
<th>Spatial Resolution in vivo</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT</td>
<td>500 micromolar (Gd-DTPA) – low millimolar (Iodine) range</td>
<td>&gt;10 µm</td>
<td>High spatial resolution</td>
<td>Patients are given high radiation doses</td>
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<tr>
<td>MRI</td>
<td>T2-contrast, iron oxide nano-particles: nanomolar-micromolar range</td>
<td>4 µm (experimental MRI), 250 µm in plane (clinical MRI)</td>
<td>High spatial resolution</td>
<td>Particle size is often large, which restricts in vivo delivery</td>
</tr>
<tr>
<td>MRI</td>
<td>T1 contrast, multilabeled targeted Gd-DTPA macromolecules: &gt;10 µM</td>
<td>4 µm (experimental MRI), 250 µm in plane (clinical MRI)</td>
<td>High spatial resolution</td>
<td>Particle size of contrast agent or reporters is relatively large</td>
</tr>
<tr>
<td>MRS</td>
<td>Millimolar range (1H at 4.7 – 11 Tesla)</td>
<td>≥0.5 cm (3 Tesla), 0.7 cm (1.5 Tesla)</td>
<td>Detection of endogenous metabolites</td>
<td>Low sensitivity results in low spatial resolution</td>
</tr>
<tr>
<td>Optical</td>
<td>Nanomolar range: ≥50 cells (fluorescence); ≥1000 cells (bioluminescence)</td>
<td>&gt;25 µm, intravitral microscopy: 1 – 15 µm</td>
<td>High sensitivity, high spatial resolution</td>
<td>Restricted depth detection</td>
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<td>PET</td>
<td>Picomolar range</td>
<td>&gt;1 mm³ (microPET), ~4-5 mm (clinical PET)</td>
<td>High sensitivity, short-lived isotopes</td>
<td>Low spatial resolution, cyclotron required for generating some isotopes</td>
</tr>
<tr>
<td>SPECT</td>
<td>Picomolar range</td>
<td>&gt;1 mm (microSPECT), ≥3 mm (clinical SPECT)</td>
<td>High sensitivity</td>
<td>Low spatial resolution, long-lived isotopes</td>
</tr>
<tr>
<td>Ultrasound</td>
<td>&gt;10⁶ microbubbles per ml blood</td>
<td>&gt;40 mm</td>
<td>High spatial resolution, cost effective</td>
<td>Few probes available</td>
</tr>
</tbody>
</table>

### Table 2: MR biomarkers currently available for applications in diseases

- **T1, T2, T2* contrast**
  - Vascular volume
  - Permeability surface area product
  - Extraction fraction
  - Vessel size
  - BOLD imaging
  - Extracellular matrix remodeling
  - Receptor expression on epithelial and endothelial cells
  - Enzyme activity dependent contrast
  - pO₂
  - pH

Spectroscopic contrast

- Total choline, phosphomonoesters, phosphodiesters
- Lactate
- Lipid
- pH
- Energy (ATP, phosphocreatine, inorganic phosphate)
- Drug pharmacokinetics
- Labeled substrate utilization

Contrast from apparent diffusion coefficient (ADC) of water

- Cell death
- Edema
- Fiber mapping

Chemical Exchange Saturation Transfer (CEST), Amide proton transfer (APT) contrast

- pH
- Receptor expression
- Gene expression

Brief outline of molecular imaging strategies

Some of the strategies in molecular imaging are broadly outlined in Figure 2 and include (a) enzyme-based reporters, (b) receptor based reporters, and (c) transporter based reporters [3].

Figure 2: Strategies in molecular imaging (from [3]).

Receptor Expression

Receptors, antigens or proteins expressed on cell surfaces provide available targets for detection and targeting. Labeled antibodies have been used to image antigens overexpressed by cancer cells, such as Her2/neu in breast cancer. MR contrast generated by injecting a biotinylated antibody that binds to a specific receptor, followed by administration of avidin linked to gadolinium-diethylenetriaminepentaacetate (GdDTPA) that binds to the biotin on the antibody [4] can be visualized on T1-weighted images.
Integrins and Angiogenesis

Angiogenesis plays an important role in cancer, stroke and cardiac ischemia. Multiple MR probes have been developed to characterize tumor vasculature. Applications of dynamic contrast enhanced (DCE) MRI based on T₁ or T₂ perfusion probes have dominated the use of MRI in the assessment of vascular parameters, such as perfusion, vascular volume, and permeability surface area product [5]. In recent advances, agents have been developed that bind specifically to receptors expressed on tumor vasculature (reviewed in [6] and [5]).

Cell tracking

Another application of MR probes has been in tracking the migration of stromal and immune cells, by pre-labeling them with gadolinium or iron-based contrast agents [7, 8]. 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 [7-9].

Hypoxia

Several noninvasive MR probes detect hypoxia. In vivo imaging of tumor oxygen consumption has been performed using 19F MRSI of 19F oxygen reporters such as 2-nitro-α-[2,2,2-trifluoroethoxy)methyl]-imidazole-1-ethanol (TF-MISO). TF-MISO is a nitromidazole-based probe that accumulates in hypoxic cells through a biochemical reduction. The reduced probes bind to endogenous cellular molecules and accumulate in hypoxic cells [10, 11] and can be used to report on hypoxia in vivo with 19F MRS [12]. Hypoxic regions are identified by imaging the uptake, distribution and retention of the probes by acquiring 19F MRSI [12] and reviewed in [6].

pH

MRS measurements of tumor pH have identified acidic extracellular and neutral to alkaline intracellular environments in cancer cells and tumors. The chemical shift of the primary intracellular inorganic phosphate (Pi) signal, detected by 31P MRS is pH-dependent and provides a measure of intracellular pH [13]. Exogenous pH markers such as 3-aminopropylphosphonate (3-APP) [14] for 31P MRS or (imidazol-1-yl)3-ethoxycarbonylpropionic acid (IEPA) [14] or 2-(imidazol-1-yl)succinic acid (ISUCA) for 1H MRSI [15] are used to measure extracellular pH. Relaxation enhanced pH measurements can also be performed in vivo using gadolinium-based contrast reagents with a pH-dependent relativity pH, such as Gd-DOTA-4AmP5-[16].

Proteolytic activity

Several proteases are involved in cancer invasion and metastases. Several new approaches have been explored to image matrix metalloproteinase (MMP) activity in vivo with MRI [17, 18] and reviewed in [6].

CEST

Chemical-exchange-dependent saturation transfer (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, [19, 20]. CEST reporter proteins can be used for molecular imaging studies in gene promoter and expression studies [21] and in detecting enzyme activity [22].

13C MRS and hyperpolarization

The hyperpolarization of spins significantly increases the sensitivity of detection of the MR signal of 13C-labeled substrates by >10,000. Hyperpolarized 13C-labeled substrates injected systemically, allow real-time metabolic mapping [23-25]. Hyperpolarized 13C has been used to
measure *in vivo* pH, and investigate metabolic pathways and enzymatic activity by labeling different substrates such as pyruvate, glutamate, or fumarate [26-28].

### List of References


