Molecular imaging in biomedical research

N. R. Jagannathan

Molecular imaging (MI) is a diverse technology that revolutionized preclinical, clinical and drug-discovery research. It integrates biology and medicine, and the technique presents a unique opportunity to examine living systems in vivo as a dynamic biological system. It is a hybrid technology that combines PET, SPECT, ultrasound, optical imaging and MR. Several MI methodologies are developed to examine the integrative functions of molecules, cells, organ systems and whole organisms. MI is superior to conventional diagnostic techniques in allowing better staging as well as to monitor the response of cancer/tumour to treatment. In addition, it helps visualization of specific molecular targets or pathways and cells in living systems and ultimately in the clinic.

Keywords: Cancer, diagnosis, molecular imaging, molecular targets, treatment.

THE last decade has witnessed an explosion of several molecular-biology techniques, amazing advances in imaging, and design of unique imaging probes. In addition, advances in stem cells, genetic engineering, nanoparticles, and immuno-histochemical techniques have provided in-depth understanding of the development of various disease processes. Despite the tremendous strides made in these areas, the cure for diseases remains beyond our grasp. Studies have shown that a disease state can be viewed as an abnormality at genetic and/or molecular level, subsequently leading to failure of multiple biochemical processes at the cellular level. The living organism is composed of cells; it can be a normal cell, or cancer cell, or about to transform into a pre-cancerous or cancerous state. Further, to localize and visualize, specific molecules are required that can localize onto or inside the cells and carry a signal. Moreover, it is necessary to look inside the cell to know its working or to see the defects that may have been caused by a disease. Many imaging techniques are appropriate for this purpose, like optical imaging, nuclear imaging and magnetic resonance (MR).

Recently, the Society of Nuclear Medicine and the Radiological Society of North America (RSNA) defined molecular imaging (MI) as 'a technique which directly or indirectly monitors and records the spatiotemporal distribution of molecular and cellular processes for biochemical, biologic, diagnostic or therapeutic applications'[1]. MI is a new concept in medicine and the fundamental idea is to probe the molecular derangements or genetic phenomena underlying such disease processes. MI is a new generation of imaging methods that provide far greater information than the conventional computerized tomography (CT), magnetic resonance imaging (MRI) or optical imaging. MI is not limited to one particular modality.

MRI, *in vivo* magnetic resonance spectroscopy (MRS), positron emission tomography (PET), single photon emission tomography (SPECT) and optical imaging (like bioluminescence) are prototype MI techniques that can be used as probes of molecular and genetic phenomena. Among these, PET and SPECT are at the forefront because it is easier to prepare the signal molecules with radioactivity. Various other modalities like MR and other imaging technologies are also useful and hold great promise.

The emerging science like nanotechnology and stem cells uses advanced MI methods to reveal invisible phenomena happening at a molecular and cellular level. For example, using MI, visualization of malignant cancer cells at the molecular level can lead to the development of minimally invasive image-guided therapies. Cancer is a complex disease and the apparent impenetrability of the disease is largely due to multiple, often redundant pathways, which appear to evolve through the genetic instability of cancer cells. The ability to identify and image key common pathways specific to cancer cells, and the ability to image the effectiveness and outcome of strategies designed against these targets are critically important in the treatment of this disease.

The purpose of this article is to give an overall picture of several MI methods that are in use today. It is not possible to give a detailed description of various MI techniques. However, the basics of the various MI methods are briefly outlined qualitatively followed by applications.

Nuclear imaging (PET and SPECT)

In PET, positron-emitting tracers like metabolically important radionuclides, i.e. isotopes (^{11}C, ^{13}N, ^{15}O, ^{18}F) are used, which are essential elements of all living organisms.

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PET is based on the following principle[2,3]. It uses the unique decay property of radionuclides that decay by positron emission. These radionuclides are produced in a cyclotron and are used to label compounds of biological interest. When such a labelled compound is introduced into the body by intravenous injection, it is distributed in tissues in a manner determined by its biochemical properties. A typical cyclotron-produced isotope decays with the emission of a positron. This positron travels a small distance in the tissue depending on both the tissue density and the kinetic energy of the emitted positron. After a series of collisions with atomic electrons from the tissue, during which the positron loses its energy and slows down, it annihilates with a nearby electron and produces two high-energy photons (with equal energy of 511 keV) emitted in opposite directions. Simultaneous detection of these photons is the basis of PET imaging. The average distance that this positron travels from its origin is small and is of the order of 0.5 mm or less. Because of thermal vibrations in the tissue, there is an additional deviation from 180°, but this effect is small like, for example, in animal systems. These photons travel with the speed of light towards the detectors positioned around the subject where they interact and get absorbed, producing an electrical signal. The absorbing material of the detector is important and determines the system sensitivity and accuracy. Typical materials used for PET are scintillates like bismuth germinate, lutetium oxy-orthosilicate and gadolinium-orthosilicate.

Similar to CT, PET and SPECT also rely on the rotation of detector arrays around the subject of investigation. With this approach, the position and concentration of a radionuclide marker introduced in the experimental animal or patient can be found. Unlike in CT, however, the emission source is unknown within the body, and rigorous reconstruction algorithms are required to increase resolution. PET has high sensitivity gain compared to other available imaging techniques. In a typical PET scan, 10^6–10^9 events (decays) will be detected. These events are corrected for a number of factors and then reconstructed into a tomographic image using mathematical algorithms.

PET has the capability to determine the concentration of molecular probes in the picomolar range *in vivo*. Spatial resolution can be improved during the image reconstruction process. Since many proteins are normally present in the nanomolar range, PET has become the method of choice for mapping and studying the function of several receptors, particularly within the central nervous system. However, the use of PET is somewhat limited for imaging apoptosis, since most positron-emitting radionuclides are physically short-lived (^{11}C physical half-life $T_{1/2}$ = 20.3 min, ^{18}F $T_{1/2}$ = 109.8 min), and must be produced using a particle accelerator, i.e. cyclotron.

The other more commonly used method is SPECT, since radioligands such as technetium (^{99m}Tc, $T_{1/2}$ = 6 h) are easier to use and less expensive to produce than the radionuclei used in PET. SPECT utilizes single photons at energies of 140 keV, instead of positron emissions which result in two 511 keV photons traversing in opposite directions. SPECT is also based on the tracer principle, but using a radioactive isotope emitting single photons rather than two photons from a positron annihilation[4]. This single photon travels through tissues and is detected on a position-sensitive detector, using similar detector technology as in PET. Because only single photons are emitted from the radionuclides, localization of the radiation source without collimators is difficult in SPECT. In clinical systems that require imaging of a large patient area, a collimator with parallel holes is used. Higher spatial resolution for a small animal imaging requires pinhole collimators. Because the photon energies involved in SPECT are smaller than PET, a number of materials can be used successfully for detector designs. Solid-state materials like CdTe have the promise to replace the traditional NaI scintillator. Small-animal-dedicated SPECT systems are also available commercially. In PET, the simultaneously perceived gamma quanta enable source localization without collimators, rendering the technique more efficient than SPECT. The useful resolution of SPECT is slightly inferior to PET, and the typical sensitivity is several orders of magnitude lower than PET. However, it is much less expensive to perform SPET scans.

Magnetic resonance

Magnetic resonance imaging

MRI is a non-invasive imaging modality that is widely used in clinical radiology for diagnosis of disease processes. MRI produces soft-tissue anatomical pictures in any desired plane, which are exquisite representations of the spatial distribution of mobile protons present in human tissues[5–7]. The soft tissue contrast resolution is superior to other currently available imaging techniques such as CT and PET. It is an indispensable imaging modality that also probes tissues and biophysical properties with exceptional in-plane resolution *in vivo*. Being non-invasive, it permits repeated measurements, thus enabling sequential data-acquisition to monitor progression or regression of disease processes as well dynamics studies.

MR is the interaction of nuclei of atoms with radio frequency (RF) field in the presence of an external magnetic field. The theory is based on the spin property of nuclei. Spin is defined as the intrinsic angular momentum of a nucleus that is responsible for the magnetic moment. Nuclei of some elements like proton (^{1}H) and phosphorus (^{31}P) behave as magnetic dipoles and are randomly oriented in the tissues. In the presence of a strong magnetic field, such as that created by a powerful magnet of the MRI scanner, these magnetic dipoles will line up either parallel or anti-parallel to the externally applied field. More spins will orient in parallel orientation, since it is the lower en-

ergy state. With the application of a RF pulse, these nuclei are flipped from a parallel to anti-parallel state. After the RF pulse, the nuclei return to the parallel state emitting RF energy. The frequency of the energy depends upon the strength of the magnetic field of the MRI scanner. By manipulating the magnetic field strength across the body, different tissues of the body can be labelled with different RFs. The sum of these frequencies is detected, sent back to the computer and analysed to produce images. For more detailed description, the reader may refer to textbooks and review articles available in the literature[5–7].

The intrinsic sensitivity of MR is low, but MRI benefits greatly from the highly concentrated water protons (~80 mol/l) in the tissue, and the fact that ^{1}H is the most sensitive non-radioactive nucleus with nearly 100% natural abundance. Most clinical MR images are from the abundant and strong signals of mobile protons present in the body, which arise primarily from water and fat. MR images are a pictorial representation of the spatial distribution of mobile protons. The densities of mobile protons present in the tissues determine the contrast in MR images together with the relaxation times.

In vivo magnetic resonance spectroscopy

MRI provides anatomical and pathological information and is a proven clinical diagnostic tool, but lacks biochemical specificity. On the other hand, *in vivo* MRS is capable of providing biochemical (metabolic) information from a well-defined region of interest, for example, the human brain[8,9]. Besides water, all living matter contains abundance of molecules with proton moieties, and if the concentration of molecules is in milli- or micromolar range *in vivo* (roughly one-tenth of that *in vitro*), it can be used as a biological MR marker.

In MRS, the data obtained are presented as spectra and are not represented as images. In fact, it is a curve or a plot (Figure 1) of MR signal intensity as a function of MR frequency measured in parts per million (ppm) relative to the frequency of the reference compound. Each peak in a spectrum derives from a different biochemical present in the tissues and its integrated signal intensity or area is proportional to the concentration of that particular biochemical. MRS can be used in a variety of normal and pathological conditions to discriminate the healthy from the diseased tissues. This gives MRS the ability to provide insight into the biochemical changes underlying a particular disease[8,9]. Information that normally requires biopsy (invasive) can now be acquired by MRS in a non-invasive manner.

MRS can be performed with a large number of isotopes. However, ^{31}P and ^{1}H *in vivo* MRS is widely used to study tissue metabolism. ^{31}P MRS probes tissue pH, energy metabolites like phosphocreatine (PCr), adenosine-tri-phosphate (ATP) and adenosine-di-phosphate (ADP) and also the precursors and degradation products of phospholipid metabolism. Using ^{1}H MRS relative levels of several low molecular weight metabolites such as choline (Cho), creatine (Cr), amino acids such as glutamate (Glu), glutamine (Gln) and N-acetylaspartate (NAA) can be measured[8,9]. Majority of applications using ^{1}H MRS on human brain demonstrate the chemical specificity and noninvasiveness of the technique in allowing metabolic fingerprinting of various pathophysiological processes[5,6,8,9]. In addition, determination of concentration of other isotopes such as ^{7}Li and ^{19}F *in vivo*, highlights the ability of MRS to monitor exogenous agents as well[5,6,8,9]. It is possible to perform MRS of other nuclei like carbon (^{13}C), lithium (^{7}Li) and fluorine (^{19}F). Natural abundance or enriched ^{13}C MRS

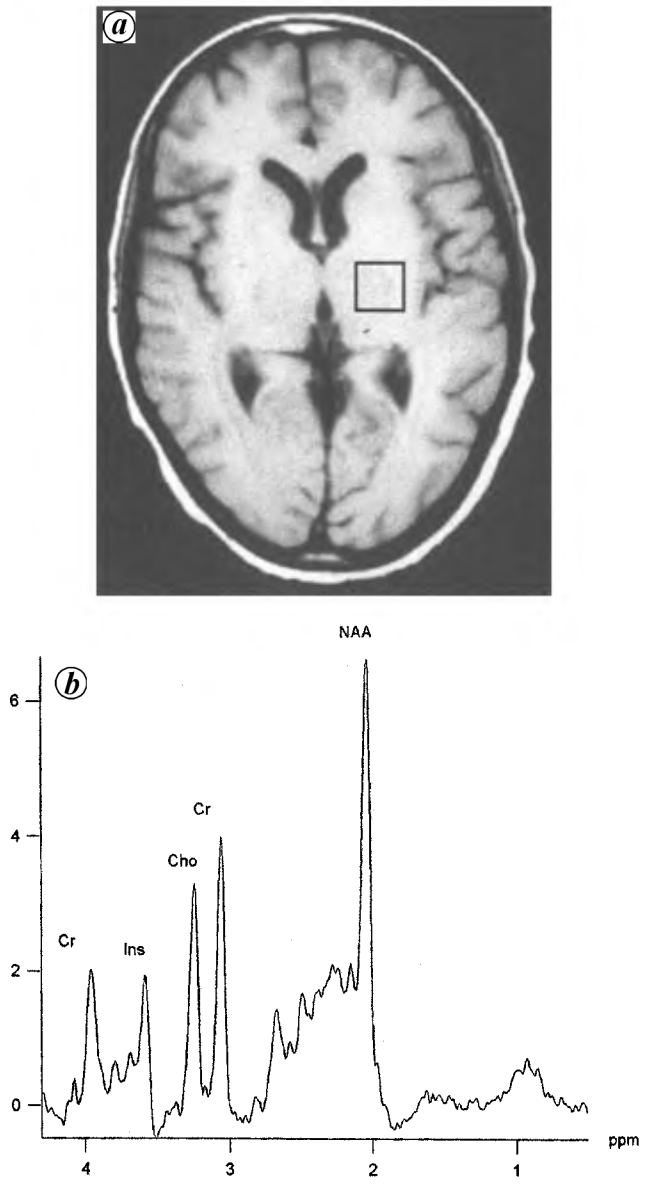

Figure 1. *a*, Axial T1-weighted MR image of a normal volunteer. *b*, ^{1}H MR spectrum from the voxel shown in (*a*).

provides information about glycolysis, gluconeogenesis, amino acids and lipids. ^{7}Li and ^{19}F MRS can be used to study pharmacokinetics[5,6].

Moreover, it would be beneficial to combine chemical specificity (obtained from nuclear imaging or MRS) with much better resolution (>50 μm) of MRI. Magnetic resonance spectroscopic imaging (MRSI) or chemical shift imaging (CSI) is a technique that combines features of both imaging and spectroscopy. This is a method for collecting spectroscopic data from multiple adjacent voxels covering a large region of interest in a single measurement. MRSI is an efficient means for comparing spectra from voxels containing different tissue types. For example, in the case of focal diseases, spectra from a lesion are compared with those from normal tissues, and heterogeneous metabolic distributions within the lesion may also be investigated. In addition, it is possible to generate a metabolic map (distribution; molecular imaging) of a particular metabolite using the MRSI technique.

Optical imaging methods

Optical imaging is an emerging field and has the potential of diagnosis of tissues *in situ* with high spatial resolution and real-time imaging[10]. In general, optical imaging includes near infrared (NIR) imaging, optical microscopy, optical coherence tomography, and light scattering and absorption. Since usable light such as that produced by fluorescence cannot penetrate more than 1–2 mm into the biological tissue, its use in biological imaging is severely compromised. However, light in the NIR range (wavelengths for 1200 mm) allows better penetration in the tissues, but with considerable absorption and scattering. Optical tomography images are reconstructed by back-projecting the transmitted light through the object along multiple paths similar to CT.

NIR imaging

Imaging using the NIR region is used to deep stage cancers, for example, breast and is characterized by a wide variety of intrinsic and extrinsic contrast agents[11,12]. The method is economical, fast, sensitive and highly portable. It has lower resolution than MRI, but NIR images can be readily co-registered with MR images, where selected voxels can be characterized with optical data for added sensitivity or specificity. NIR imaging also has high sensitivity for very low contrast agent concentrations. Contrast is based upon intrinsic signals of blood concentration or blood pooling related to angiogenesis and of blood endoxygenation due to hyper-metabolism. Extrinsic contrast is imaged with tricarbocyanine probes related to tumour hyper-metabolism and extrinsic blood pooling agents, similar to gadolinium chelates of MRI. A wide variety of NIR-targetted probes can be delivered in a 'cryptic' form and decoded by tumour-specific enzymes to an optically detectable form (i.e. molecular beacons). These probes also may signal gene expression by tagging the conventional markers (e.g. β-galactosidase).

Optical microscopy

Optical microscopy includes scanning microscopy and spectroscopic microprobes (e.g. Raman, elastic scattering and fluorescence)[12,13]. Confocal and multiphoton confocal microscopy is used to obtain depth information or to enhance imaging in optically scattering tissues with high image resolution. It yields enhanced imaging depth and enables living cell imaging with reduced photobleaching effects. Clinical application requires development of endoscopic mediation[14].

Optical coherence tomography

Optical coherence tomography (OCT) provides cross-sectional images of the tissue *in situ*[14] and relies on detecting scattered light and the depth of imaging on detecting scattered light; the depth of imaging is limited to within 2 to 3 mm of the surface in most tissues. It gives 'histology-like' images with resolution as high as 5 to 10 μm in real time, with no need for excision. Thus, this technique has the potential for diagnosis of early neoplasia and surgical guidance. It is largely still a research technique.

Light scattering and absorption

Two other types of optical imaging methods based on absorption and scattering of light of tissues have emerged[15–17]. Extensive studies on *in vitro* and *in vivo* optical properties of human tumours show increased absorption due to angiogenesis that may be related to hormonal perturbation of the population of cell-like organelles, mitochondria, etc.

Light scattering methods provide quantitative characterization of tissue optical properties (absorption and scattering), and quantitative measurements of endogenous biochemical constituents like tissue haemoglobin concentration, oxygen saturation, water and fat. In addition, quantitative measurement of exogenous probes and drugs is possible. Photon migration techniques give imaging of deep (i.e. several centimetres) tissues by taking advantage that light at NIR wavelengths is not highly absorbed by the tissue and thus can penetrate several centimetres. Multiple scattering of light degrades image information; therefore, most of these techniques focus on either low-resolution imaging or functional assessment of tissue at low resolution. These techniques are still in experimental stage.

Light absorption methods include fluorescence imaging and spectroscopy. With this approach and with photoacoustic imaging, endogenous tissue fluorescence and

exogenous contrast probes can be used to localize tumours, characterize tissue biochemical environment, and quantify flow and extravasation kinetics. Spectral imaging, which represents a hybrid optical diagnostics, obtains spectroscopic information and renders it in image form. It is also possible to combine spectroscopy with photon migration in order to perform functional assessment of deep tissue structures. One example is the spectroscopic detection of oxy- and deoxy-haemoglobin for noninvasive assessment of tissue oxygenation.

Bioluminescence

In addition, biological processes of intact organisms can be studied by another optical MI technique called bioluminescence. By harnessing the natural light-emitting properties that allow fireflies to flicker in darkness, bioluminescence imaging (BLI) can be used to track cancer cells, bacteria and numerous other processes[18]. BLI is based on the sensitive detection of visible light produced during enzyme (luciferase)-mediated oxidations of a molecular substrate when the enzyme is expressed *in vivo* as a molecular reporter. Bioluminescence can be used to image as deep as several centimetres within the tissue, which allows organ-level resolution. There are several bioluminescent systems, each requiring a specific enzyme and substrate. The most commonly used bioluminescent reporter is luciferase from North American firefly (*Photinus pyralis*; FLuc). Other luciferases that have been cloned are from jellyfish (Aequorea), sea pansy (Renilla, R Luc), corals (Tenilla), click beetle (*Pyrophorus plagiophthalamus*), and several bacterial species[18].

Applications

Today's challenge in medical science is the ability to detect a small number of cells that are at the early stage of the disease. Advances in human genome research led to the discovery of early disease indicators called biomarkers. Translation of this knowledge into aspects of disease expression inside the body is an exciting aspect of MI[2,10,19,20]. Imaging agents that look at the ability of cells to grow (or proliferate), to die (widely known as apoptosis or programmed cell death), or to form new blood vessels (such as angiogenesis) are part of untapped future potentials of MI. The various MI methodologies available today can be used to study most organs of the body and have contributed to our understanding of the basic physiology and pathophysiology of oncological disorders, the brain, the heart, and other organs.

For example, PET plays a major role in the development of new stable (nonradioactive) drugs and is an ideal tool to image phenotypic alternations resulting from altered genotype. To date, the most common application of PET in routine clinical studies has been in patients with cancer, since PET images show functional alterations caused by molecular changes in contrast to the traditional imaging methods like CT[2,3].

Further, to understand and exploit molecular pathways in cancer or in any other disease process for therapeutic strategies, it is essential to detect and image the expression of these pathways and to determine the impact of this expression on function at the cellular level, as well as *in vivo*[10,19,21]. Transgenic tumour models are developed to understand cancer. For example, transgenic cells or animals whose genetic composition has been altered by addition of foreign DNA, or transgene, can be studied using MR and other MI techniques that provide unprecedented opportunities to understand and characterize specific targets and pathways in cancer.

Using small-animal PET systems excellent temporal resolution, good sensitivity and whole-body coverage can be achieved that would enable imaging protocols that were not possible in the past. Further, studies on transgenic cells and mouse models will be useful to determine the consequences of over-expression, under-expression or complete inactivation of the gene under investigation[2,3,10,19]. These studies enhance the understanding of the function of genes and how they influence the malignant phenotype. Information derived from such transgenic models also leads to the development of therapies for cancer treatment. In addition to the more traditional imaging of xenograft tumour models or other regions of interest specific to protocols, one can now perform first-pass angiography or look at whole-body pharmacokinetics.

Further, experimental animals can be grown with a disease that is marked genetically in such a way that the cells produce a molecule that emits colour (typically fluorescent). Using optical imaging methods it is possible to evaluate the effect of new drugs or treatment on these cells *in vivo*. Many pharmaceutical and biotechnology industries are carrying out research to speed up their drug-discovery approaches using several MI methodologies[22].

Diagnosis of cancer at the early stage is a challenge. Conventional diagnostic techniques are unable to detect such miniscule tumours, and by the time many cancers are diagnosed, metastases cells may have already begun to migrate from the primary site. Moreover, in cancer detection, sub-centimetre metastases that are missed by conventional, anatomically based imaging methods may be detected in patients by MI methods. For example, PET imaging with ^{18}F-flurodeoxyglucose (FDG) is widely used for diagnosis, staging and detecting cancer and other recurrent diseases[2,3]. Figure 2 presents the non-Hodgkins lymphoma used for staging. The coronal PET–CT image shows intense FDG uptake in retroperitoneal lymph nodes and right axillary lymph nodes. Figure 3 is the PET–CT scan showing intense FDG uptake in the right breast and right axillary lymph nodes of a patient suffering from carcinoma of the breast.

Using nanoparticles it is possible to detect cancer metastases in non-enlarged nodes as small as 1–2 mm in size. Along with other biomarkers and emerging molecular tools like DNA screening, tissue proteomics and metabolism analysis, and serum markers, this information soon may be used for screening, diagnosis, detection of recurrence, and treatment assessment. In fact, MI has changed the cancer staging procedure. In future a micro-fluidic chip would show a person's protein fingerprint simply using a drop of blood, and this fingerprint would reflect the person's health condition.

Both PET and MR are useful to study and characterize cardiovascular systems[23,24]. Diagnosis and characterization of coronary artery disease can be made by measuring blood flow and its response to physiological and pharmacological stresses[23,24]. In addition, PET imaging of the brain has helped to measure cerebral glucose metabolism, blood flow, enzyme activities, neurotransmitter synthesis and receptor binding in a host of neurological and psychiatric disorders.

MI techniques are increasingly used in the management of patients undergoing therapy[5,6,25–29]. For example, in breast cancer it is possible to use choline metabolite or water-to-fat ratio from *in vivo* MRS as a marker to monitor the response of patients undergoing treatment[28,29]. Similarly, PET with FDG can be used for accurate tumour volume detection. Such studies are promising for the assessment of response following chemotherapy and radiotherapy[25]. The latest technical development has been the introduction of combined PET/CT scanners. In this approach anatomical information is obtained using X-rays, and, immediately afterwards, with the patient in the same position, FDG–PET is carried out to acquire functional information. This alleviates the difficulty in multimodality image processing or co-registration, as shown in Figures 2 and 3. PET/CT has higher sensitivity and specificity than either technique alone. Recently, development of PET/MRI is underway that will provide even better spatial/anatomical resolution than is achieved using the PET/CT system.

In recent times another methodology called 'molecular MRI' is making advances in identifying the functional and metabolic changes that precede anatomic evidence of the disease, giving drug delivery and effectiveness and monitoring basic cellular processes[30–33]. Since conventional MR is not sensitive in detecting molecular markers, use of high-field MRI has increased since MR signal increases with field. Work on molecular contrast agents facilitates early diagnosis and outcome control.

Numerous applications are based on marker molecules[31–33]. Recently, it has been shown that MRI contrast can rationally be adjusted with good biocompatibility by chemically malleable marker ligands linked to magnetically active compounds, such as super paramagnetic iron oxide nanoparticles (SPIO) or gadolinium (Gd) chelate, that have been used to detect the expression of certain re-

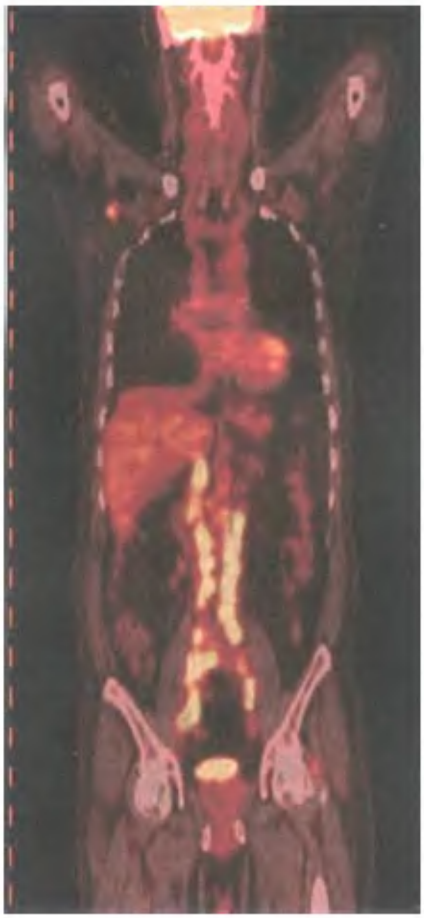

Figure 2. Non-Hodgkin lymphoma for staging: Coronal PET–CT image shows intense FDG uptake in retroperitoneal lymph nodes and right axillary lymph nodes.

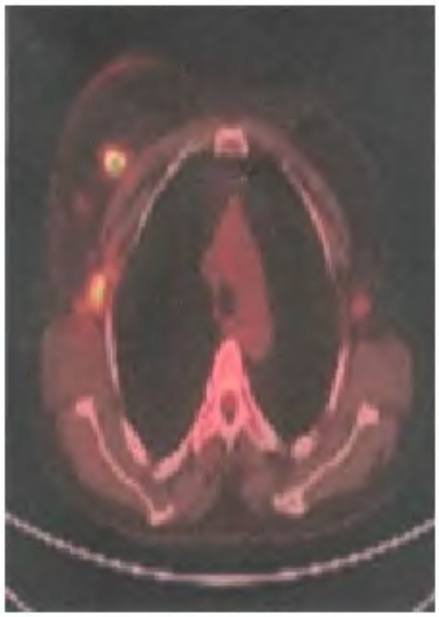

Figure 3. PET–CT scan showing intense FDG uptake in the right breast and right axillary lymph nodes.

ceptors in tumours. Research on VSUP (very small super paramagnetic iron oxide particles), USUP (ultra superparamagnetic particles), and other such compounds are being actively pursued[30].

Designing targetted imaging agents for MRI requires tricky chemical engineering[21]. Moreover, huge quantities of magnetic material have to bind to a target in order to generate a strong enough signal for MRI to detect. Recently, it has been shown that 200 nm droplets of inert perfluorocarbons can be used as molecular pincushions to hold a payload of up to 100,000 Gd molecules[32]. To this, a smart drug can be attached that clamps onto a receptor. This drug helps visualize tumours as small as 1 mm in diameter on MRI. Nanodroplets also can be used to deliver concentrated doses of the chemo drug directly to tumours, bypassing healthy tissue. Further, use of nanoparticles may help MRI to detect or visualize tiny clumps of tumour cells that may have spread to the lymph nodes. The nanoparticle may consist of balls of thousands of iron molecules or similar agents held together with a sugary coating. About half the size of a virus, the nanoparticles are sucked up by healthy lymph nodes, but not by the cancerous ones. Studies on prostate cancer patients showed that nanoparticles enabled MRI to spot more than 95% of lymph-node metastases (confirmed later by biopsies). However, conventional MRI could detect only few lesions.

Development of new tracers that target specific biological properties of cancer cells, including ^{11}C-acetate and ^{18}F-fluoroethycholine for lipid synthesis and ^{19}F-fluorothymidine (FCT) for assessing DNA replication and cell proliferation has been explored using PET[2,3].

In vivo MR spectroscopy (MRSI or metabolic mapping) also characterizes tissues by their metabolic signatures for tissue diagnosis and evaluation of therapeutic response. Despite the recent advances of new methods to image gene expression *in vivo* in transplant tissues and using positron emitting radioisotopes, MRI and MRS have the greatest potential for use in non-invasive gene transfer assessment. Recent advances in MRSI technology also permit real-time noninvasive imaging of gene expression *in vivo*[10,33]. This technology would enable temporal monitoring of gene expression in living animals during the development of disease or during administration of transgenes for gene therapy, and eliminate the need for animal-intensive and labour-intensive studies. If gene therapy in humans becomes a reality, then monitoring of gene transfer efficiency and expression in clinical settings would require noninvasive techniques. Therefore, an obvious need exists for noninvasive tools to measure the efficacy of gene transfer. In addition, parameters that reflect molecular mechanisms such as blood flow, metabolism, and proliferative activity provide clinicians more information to aid in characterizing the pathology.

It is known that MRS is a useful, noninvasive technique for measurement of metabolic status of tissues without the use of ionizing or nephritic agents. It has the added advantage that spectral information is obtained in minutes and can be used to determine enzymatic rates *in vivo*, quantitatively. In addition, incorporation of techniques aimed at significantly decreasing the time required to obtain high-quality spectra using 2D and 3D spectroscopic imaging (MRSI) techniques, should provide tremendous boost to the imaging of metabolite levels spatially in the tissues that would allow improved characterization of heterogeneous lesions[34]. For example, normal prostate contains high level of citrate which can be mapped in a normal volunteer (Figure 4) using 3D MRSI technique. In prostate carcinoma patients[34], the citrate level decreases while the choline level increases (Figure 5 *b*, and the corresponding molecular imaging of choline level is shown in *a*). Similar application of MRSI in breast cancer is shown in Figure 6, where choline level is higher than the normal breast tissue. Further, increased availability of 3T, 4T and even 7T MRI scanners should further enhance the spectral quality, particularly in proton (hydrogen) MRS studies.

Imaging of apoptosis, hypoxia, and blood flow is possible with the development of new tracers and ligands[35]. The methods are divided into groups by the timescale of detection and the detection targets. The methods used traditionally are microscopic in nature. Techniques like *in vitro* NMR, MRI, *in vivo* MRS, nuclear imaging, optical imaging and even ultrasound are used. Success of these techniques lies on the myriad of changes involving membrane composition, protein synthesis, glycolysis, phosphatidylcholine, phosphatidylserine and cell fatty acid turnover, energy levels and even intracellular pH throughout the execution of the 'apoptotic programme' *in vivo*. Apoptosis imaging also has become an important tool in selection, e.g. non-responding patients form responders much earlier than is currently possible from anatomical imaging techniques only. Likewise, these techniques could prove helpful in drug development and pre-clinical testing[22].

As discussed earlier, superficial structures with micron-level spatial resolution can be imaged using various optical imaging methods[10,11,36–38]. Such high-resolution imaging can be sensitive to cytologic and morphologic changes, extracellular matrix structure and composition, tissue dysplastic transformation, blood flow and other parameters. Optical imaging can detect pre-neoplastic and early neoplastic changes, which is important since treatment is difficult once invasive carcinoma and metastases develop. Another major application is the guidance of surgical intervention and real-time assessment of tissue response to treatment. This technology provides more precise guidance of surgical intervention by aiding in the determination of tumour margins or by facilitating surgery on or near important normal structures, such as nerves and blood vessels.

BLI technology is better than standard assay techniques that require biopsy and necropsy tissues[18]. For example,

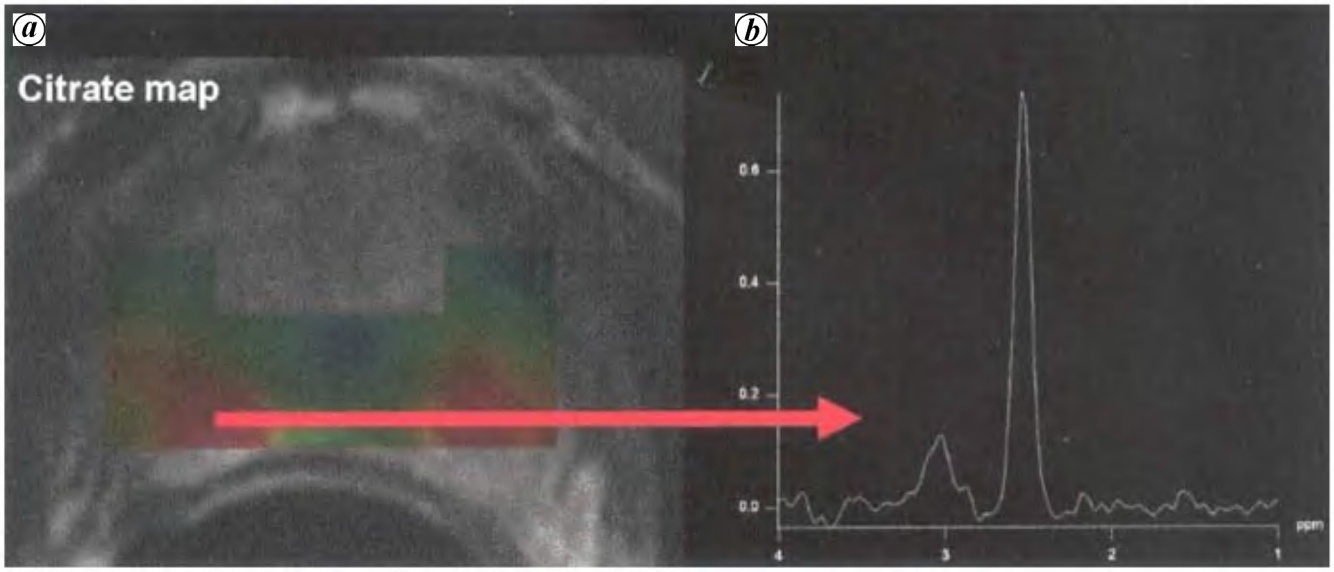

Figure 4. *a*, Citrate metabolite map superimposed on T2-weighted MR image of prostate of a normal volunteer. High concentration of citrate is shown as red. *b*, ^{1}H MR spectrum from a citrate-rich region of prostate.

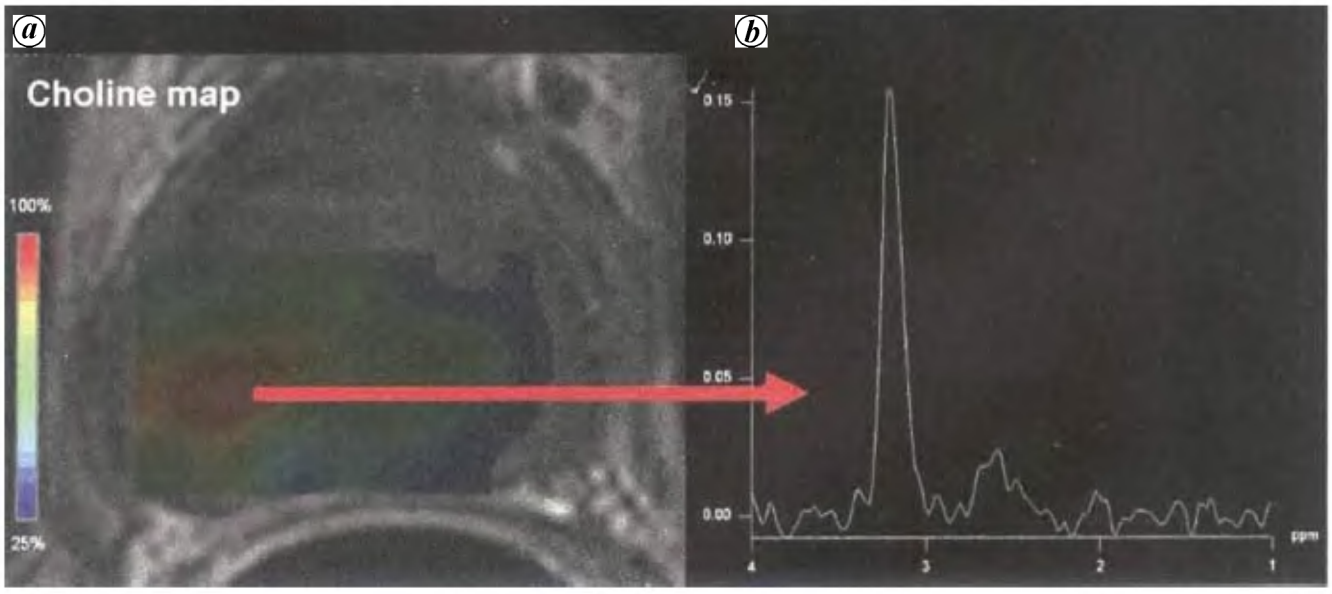

Figure 5. *a*, Choline metabolite map superimposed on T2-weighted MR image of prostate of a patient with prostate cancer. High concentration of choline is shown as red. *b*, ^{1}H MR spectrum from a cancer region of prostate having high concentration of choline.

by tagging cells with the luciferase gene, cells can be followed throughout the body by means of a digital camera. BLI can be used to detect biological changes *in vivo* for example, it enables to study disease progression, response to therapy, growth of new cells and tissues. BLI has been applied to monitor transgene expression, progression of infection, tumour growth and metastasis, transplantation, toxicology, viral infection and gene therapy. It is also a useful optical imaging technique for mammalian tissues because these tissues have low intrinsic bioluminescence; therefore, images can be generated with high signal-to-noise ratios.

In addition, BLI being a simple technique can be used to monitor the progression of the disease, and allows localization and serial quantification of biological processes without killing the experimental animal[18]. Thus the number of animals required for experimentation is reduced and multiple measurements can be made on the same animal over time, minimizing the effects of biological variation.

Studies carried out till date have shown that optical imaging methods are promising and are in the transition from laboratory studies to early phase clinical investigations. The modalities are developing rapidly and have the potential for high spatial and temporal resolution, and are

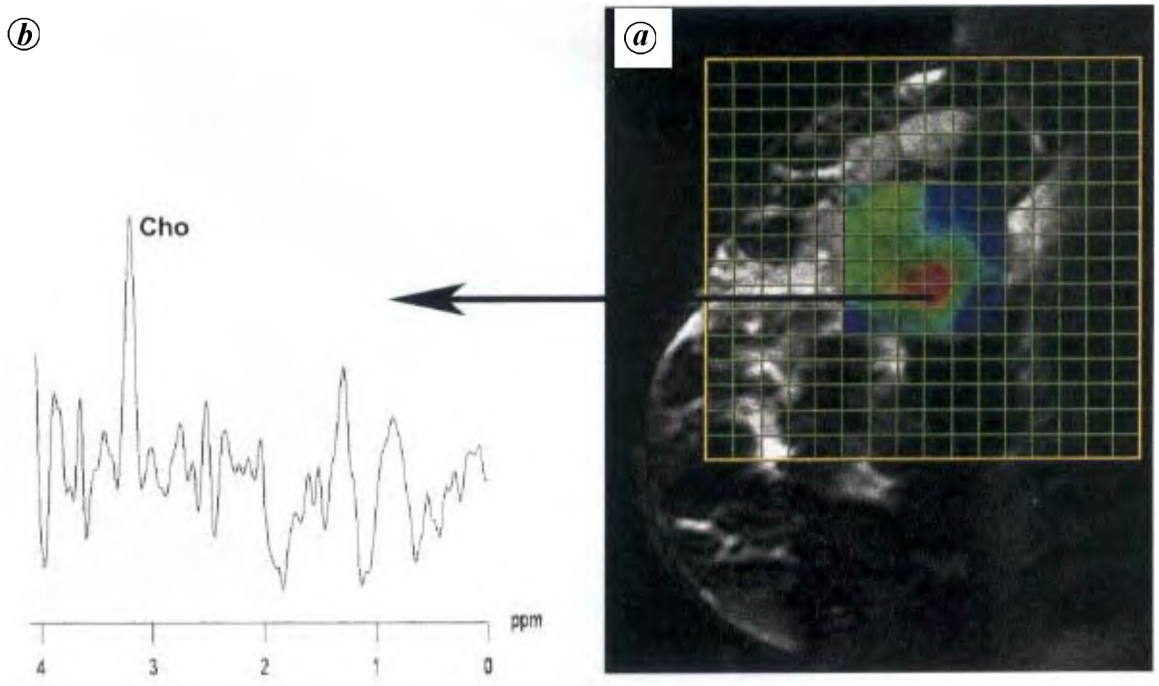

Figure 6. *a*, Choline metabolite map superimposed on T2-weighted, fat-saturated MR image from breast of a cancer patient. High concentration of choline is shown as red. *b*, ^{1}H MR spectrum from a voxel positioned in the tumour region of breast showing high level of choline.

free of ionizing radiation. Limitations include lack of commercial instruments, lack of suitable methods for tissue characterization *in vivo*, and limited access to deeper parts of the body due to the limited pathlength of most optical wavelengths in the tissues.

Optical imaging is unique in having the advantage of a very high (i.e. picomole) sensitivity and speed for cryptic and non-cryptic molecular beacons, thus affording a unique complement to MRI, PET and SPECT. Another advantage of the optical method lies in its simplicity, affordability and portability. The future research should focus on exploiting the targettable probes and molecular beacons for deep tumours *in vivo*, thereby combining many of the advantages of radioactive labels or specific biochemical markers.

Summary

MI techniques have a broader scope than simple preclinical animal research or a single imaging modality. This methodology is relatively new and can be used for early diagnosis, staging, monitoring the assessment of disease and its response to various drugs. In future, MI is expected to improve the accuracy of identifying disease targets and develop specific MI probes that bind to these targets. Such targets are expected to provide the status of the disease process earlier, help identify the processes that initiate it and cause progression, and to distinguish between aggressive and indolent states. Once a molecular target, its affinity ligand and an imaging system have been identified, the next step is to synthesize a corresponding molecular agent for human use. It will dominate and influence the daily practice of medicine for years to come, and contribute to improved patient care from diagnosis to therapy. With such advances, medicine would become be predictive, personalized and preventive.

In developed countries, most universities and medical institutes have a dedicated centre for MI, while in developing countries no such dedicated centre/s exists. The need is to establish centres of excellence on MI to initiate research on several diseases that are unique to these developing countries.

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Scoping technology options for India's oil security: Part I – ethanol for petrol

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Crude oil prices recently crossed US$ 75/bbl, fuelling serious concerns whether India's rapidly expanding economy can sustain a high and growing level of crude imports. There are also serious concerns of global warming from burning of fossil fuels. It may be time for India to explore options which can substitute petrol and diesel and are climate-friendly. In a series of two articles, we examine a few such technology and policy options. Part I focus on options for substituting petrol by ethanol from sugarcane: molasses, sugarcane juice and cellulose (bagasse). Part II analyses options for diesel substitution: Fischer–Tropsch liquids from coal, and bio-diesel from oil-bearing plants like jatropha.

Keywords: Ethanol for petrol, India's oil security, scoping technology.

THE last few years have been critical for global oil consumers. After about 150 years of growth, the world started consuming more crude oil than it could discover. About 944 billion barrels of oil has so far been extracted, and about 1200 billion barrels remain underground[1]. With the present rate of production, about 81 million barrels a day[1], the reserves would last for only 40 years, though the numbers vary depending on whose data we trust. Oil-producing countries like Saudi Arabia inflate their data on reserves and talk of almost unlimited oil, with no end in sight for at least 60 more years. Even the US government data include all oil lying under the Arctic tundra and possibly under Antarctica, without worrying either about the cost of production or the environmental price. Even with all these reserves, the peak in oil production is expected anytime between 2006 and 2020. The fear of depletion is already seen with nations scrambling at each other to sign long-term supply contracts with oil-producers. US$ 100 a barrel, unthinkable just a few years ago, appears to be a distinct possibility.

Predicting the exact year when the world will 'run out of oil' is not going to be easy or even necessary. However, based on available statistics and experience in predicting the discovery and drying of oil wells, the end of oil as an abundant and cheap energy resource is near. Even if supplies of oil were to continue for several more decades, there is increasing pressure to look for alternate fuels in response to the potential threat of global warming. It is time for consumers and the global economy to move away from the crude oil-based economy to other fuels.

This scarcity comes at an inconvenient time for rapidly growing economies like India or China, whose elasticity of energy consumption to GDP is more than 1. The Indian economy is showing robust growth of around 8%. By 2040, one in five Indians is expected to own a car as against one in 100 now[2]. India is likely to account for 15% of the world's oil demand[2] by 2040. India's domestic production of crude has almost stagnated at around 11 million tonnes per annum. Crude imports touched 90 million tonnes in 2003–04 and will only increase further[3]. Unlike developed countries, India is a diesel-based economy. Diesel consumption is about five times that of petrol. Diesel is the fuel for trucks, agricultural machinery, water pump-sets and stand-by generators[4].

Given India's ambitious growth targets, volatility of crude oil prices and concerns of climate change, this may be the time for India to explore substitutes for diesel and petrol. Technology analysis in and of itself is not meaningful without the context of economics, policy and overall sustainability (including environmental). In this analysis, we examine these aspects for:

- Ethanol from sugarcane substituting petrol;
- Diesel from coal using Fischer–Tropsch synthesis;
- Bio-diesel from oil-producing seeds such as jatropha.

We have not examined hydrogen as a transportation fuel here. Hydrogen technology is many years away from even modest adoption, especially if envisaged with alternative prime mover technologies such as fuel cells. It also involves a radical change in the supply-chain. Hydrogen is not a primary fuel but a carrier; it requires primary fuels for production, which remains an energy supply concern. Much of the hydrogen used today comes from

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