

ARTICLE

Molecular imaging in oncology

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Abstract

Cancer is a genetic disease that manifests in loss of normal cellular homeostatic mechanisms. The biology and therapeutic modulation of neoplasia occurs at the molecular level. An understanding of these molecular processes is therefore required to develop novel prognostic and early biomarkers of response. In addition to clinical applications, increased impetus for the development of such technologies has been catalysed by pharmaceutical companies investing in the development of molecular therapies. The discipline of molecular imaging therefore aims to image these important molecular processes *in vivo*. Molecular processes, however, operate at short length scales and concentrations typically beyond the resolution of clinical imaging. Solving these issues will be a challenge to imaging research. The successful implementations of molecular imaging in man will only be realised by the close co-operation amongst molecular biologists, chemists and the imaging scientists.

Keywords: *Molecular imaging; cancer; oncology.*

Introduction

Cancer is a genetic disease that manifests as an abnormality in cellular processes of replication and metabolism^[1,2]. A combination of technological development and biological insight has propelled cancer research and treatment into the molecular domain. Most novel anticancer therapies are now directed against specific molecular targets known to influence key cellular processes. The sequencing of the human genome^[3,4] has significantly facilitated this process with microarray analysis^[5], bioinformatics^[6] and high-throughput screening^[7,8] providing additional insight into the molecular basis of cancer.

Imaging these molecular events and their modulation in man will be a key enabling process in the delivery of modern cancer care. The new discipline of molecular imaging (MI) therefore seeks to 'image molecular events *in vivo*'^[9] often with cell and phenotypic mouse imaging added to this definition^[10]. Biomedical imaging has already played a key role in defining many cellular and biochemical mechanisms *in vitro*^[11] and is substantially influencing drug development^[12]. The translation of

MI to *in vivo* and clinical imaging provides the next challenge. Achieving this will require the integration of chemistry, molecular biology and imaging hardware. The ultimate aim is to establish these practises in man. Although MI represents a new discipline rather than a new science its language remains unfamiliar to most imaging clinicians and scientists. The vocabulary of chemistry and molecular biology needs to now join that of the clinical imaging sciences^[13,14]. MI has already had an impact on strategic thinking in radiology. The National Cancer Institute^[15,16] and National Institute of Health^[17,18] are supporting the development and teaching of MI in radiology^[19].

An approach to MI

Length scales and sensitivity

Between four to six orders of magnitude separate cellular and molecular events with nanomolar concentrations characterising cellular metabolic processes. Small molecular weight compounds (<1000 D) typically

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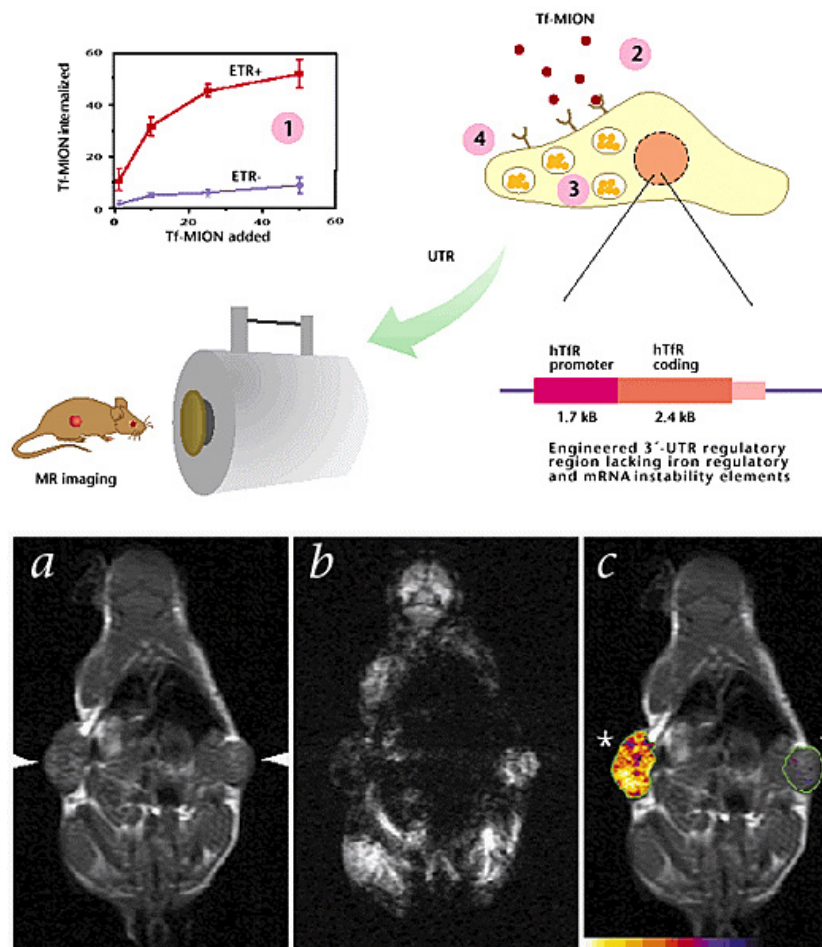


Figure 1 Gene transfection demonstrated via MR. Tumour cells were genetically engineered to overexpress the transferrin receptor (a cell membrane receptor involved in regulating cellular iron uptake). Iron in the form of monocrystalline iron oxide nanoparticles (MIONs) therefore accumulated in tumour cells overexpressing the transferrin receptor. The MIONs induce a high susceptibility (the left-hand tumour in these animals—the control tumour is on the right flank) imaged as signal loss by MR. The images were acquired on a clinical 1.5 T system. Reproduced with permission^[30]. Copyright Nature Publishing Group.

have molecular radii of 10^{-10} m compared with the radius of a cell, 10^{-5} m. By comparison a clinical magnetic resonance (MR) scanner has an in-plane spatial resolution of the order of 10^{-3} m. Positron emission tomography (PET) is sensitive to 10^{-9} M whilst magnetic resonance spectroscopy (MRS) at clinical field strength is 10^{-4} M. Successful MI therefore requires^[9,10] probes with (i) high affinity and specificity, (ii) the capability of overcoming biologic delivery barriers, (iii) amplification strategies and (iv) sensitive, fast and high resolution imaging techniques.

The MI 'probe' concept

Independent of imaging modality molecular probes can be classified into three types^[10]: (i) the compartmental probe; (ii) targeted probes; and (iii) 'smart' sensor probes. Compartmental probes typically assess physiological

parameters (i.e. flow and perfusion). Targeted probes on the other hand specify a molecule, receptor or enzyme of interest and an imaging component that provides the physical contrast. It is this specificity that makes the probe molecular and small molecules, peptides, enzyme substrates and antibodies have all been used in this manner. Finally, 'smart' probes are agents designed to activate exclusively in the presence of their intended target. The absence of a significant background signal gives smart probes a substantial signal advantage over simple targeted agents. Nanosensors, for example, are a recent addition to this group^[20,21].

Limitations and developments in the probe concept

A high specificity agent is useless if it cannot reach its target and limitations in delivery due to inherent biolog-

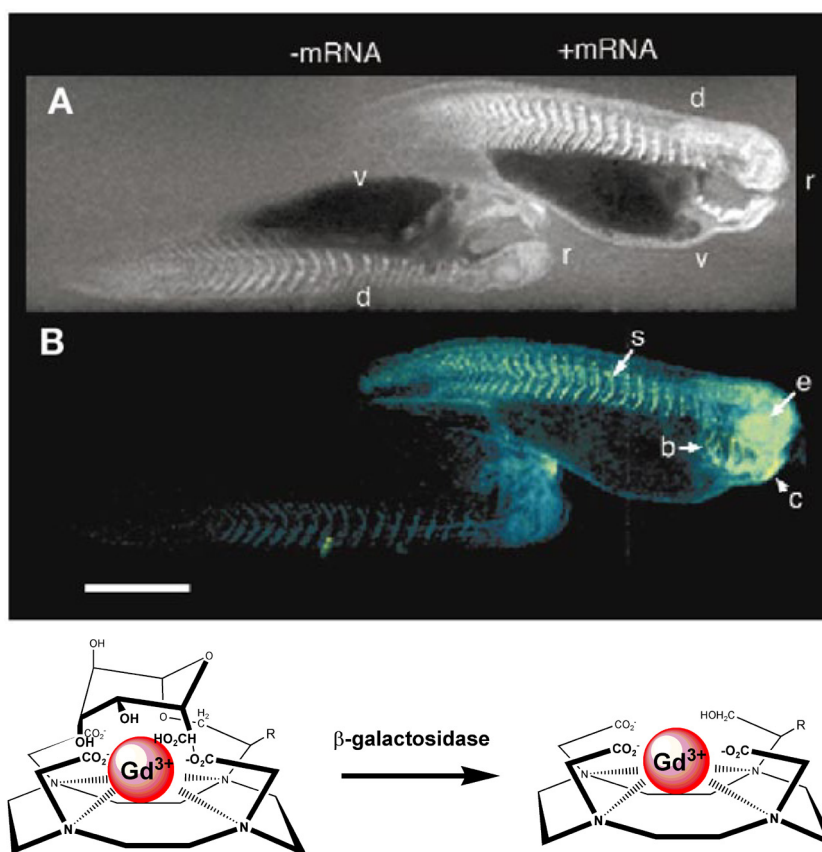


Figure 2 A ‘smart’ MR contrast agent used to demonstrate gene expression. In the presence of β -galactosidase expression the β -galactopyranose ring protecting the Gd^{3+} is cleaved allowing bulk water access to the paramagnetic ion. The images labelled A and B are of *Xenopus* (African claw toed frog) embryos that either do or do not express β -galactosidase. The embryo labelled +mRNA is expressing β -galactosidase and significantly more detail is seen in this embryo than in the one labelled -mRNA. The bottom embryo is oriented upside down compared to the top one. Reproduced with permission^[32]. Copyright Nature Publishing Group.

ical barriers^[22,23] is an important issue for all imaging modalities. Possible solutions^[10] include (i) peptide membrane translocation signals, (ii) polyethylene glycol (PEG)-ylation, (iii) local delivery and (iv) development of long circulating compounds which provides a more homogenous agent distribution. Other than radionuclides with their inherently high detection sensitivity some signal amplification strategy is required. Amplification strategies^[10] include (i) improving target concentration and (ii) probes that alter their physical behaviour through interaction with the target. These include agents that alter in relaxivity whilst much work has been done with fluorescence dequenching in optical imaging.

The tumour microenvironment—an opportunity and challenge for MI

The temporal and spatial heterogeneity of tumours is well recognised^[24]. The result is that a tumour’s chemistry,

biology and physical characteristics vary as a result of its natural history and importantly following therapeutic intervention. The basis of this heterogeneity is a constant interaction between cancer cells, the extracellular matrix, host immune cells and vasculature. As a result oxygen tension, tumour pH, metabolic status and the tumour genotype are interdependent. This constant alteration is characteristic, permitting a tumour to survive in the face of a host and therapeutic response. Several imaging strategies are currently focused on probing this complexity *in vivo*. Historically *in vivo* imaging has concentrated on morphological anatomy, which is known to poorly reflect underlying biology^[25]. Recently, apoptosis and angiogenesis, both important mechanisms in cancer, have been the focus of imaging efforts^[26]. The receptor–ligand interaction is the generic mechanism common to these pathways with imaging seeking to exploit this interaction. Receptor–ligand interactions form the basis of the majority of biological signalling with inhibition being a common therapeutic goal.

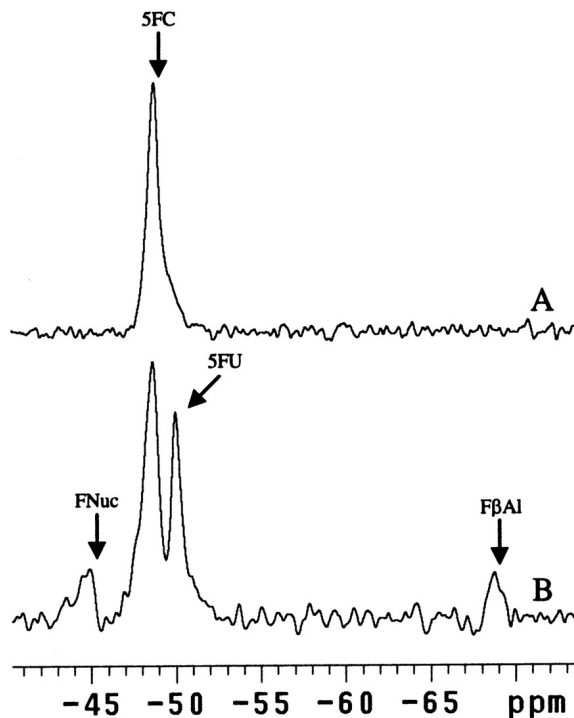


Figure 3 Demonstrating *in vivo* gene expression by ^{19}F -MRS. Using cytosine deaminase (CD) transfected cells (CD is a fungal gene and has no mammalian counterpart) tumours were grown on the flanks of experimental animals. CD catalyses the conversion of 5-fluorocytosine (5FC) into the active anticancer drug 5-fluorouracil (5FU). The resonant frequency of 5FU is different to 5FC allowing its identification *in vivo* and confirming transfection with the CD gene. Fnuc, fluoronucleotides; F β AI, fluoro-beta-alanine. Reproduced with permission^[33]. Copyright (1999) National Academy of Sciences, USA.

Specific examples of MI—a modality-based approach

MI remains predominantly focused on preclinical disease models. Research has principally focused on (i) imaging gene delivery and exogenous marker genes and (ii) the imaging of molecular pathways such as angiogenesis and apoptosis.

Nuclear magnetic resonance

Magnetic resonance imaging (MRI) has high spatial resolution but an inherently low signal yield necessitating signal amplification strategies^[27]. In the field of angiogenesis, targeted agents against the $\alpha_v\beta_3$ -integrin (these are transmembrane proteins involved in cell-cell adhesion) have demonstrated tumour neovasculature to advantage in a rabbit VX2 tumour^[28]. In this approach the targeting moiety was an engineered antibody against

$\alpha_v\beta_3$ attached to a liposome carrying several Gd(III) moieties (in the form of chelates) which increased the relaxivity of the agent. The issue of barrier penetration was minimised because the molecular target was de facto intravascular. Targeted agents have also been described against apoptotic markers^[29] and transgene (Fig. 1) products^[30,31].

Several 'smart' MRI contrast agents have been described. Perhaps the best known is EgadMe^[32] in which a galactopyranose ring protects a Gd(III) ion from bulk water (Fig. 2). In the presence of β -galactosidase the ring is cleaved allowing access of bulk water molecules to the Gd(III).

MRS is a technique particularly suited to probing molecular events. The use of MRS is discussed later in this article but suffice it to say that the technique has successfully been used to demonstrate gene transfection in several *in vivo* model systems^[33,34] (Fig. 3). This is particularly encouraging given the recent developments in the use of gene-directed enzyme prodrug therapy in cancer^[35].

Computed tomography (CT)

Although not as prevalent as clinical CT this modality has a role in MI. In comparison to PET and MR a small animal CT is cheap. It has a resolution of the order of 50 μm , unlimited depth penetration and a fast scanning time (50 μm in-plane resolution can be achieved in the order of several minutes compared with several hours via MR). Soft-tissue tumours, bone and lung are well suited to study via CT. Small animal CT has been particularly avidly applied to phenotyping the myriad of knock in/out mice^[10] which supports preclinical work in cancer.

Ultrasound

Ultrasound has the advantage of real-time imaging with down to 40 μm in-plane resolution at 5 mm depth using 20–60 MHz probes. Bone and air artefacts notwithstanding, ultrasound can be a useful technique when physiological information on flow is desirable. Recent developments in hardware and targeted agents have allowed ultrasound to translate to imaging molecular processes *in vivo*^[36,37]. The targeted agents are based on the same premise as in other modalities, in this case the imaging contrast being provided by microbubble technology. Improved sensitivity through the use of harmonic imaging^[38] has further improved the signal to noise characteristics of ultrasound.

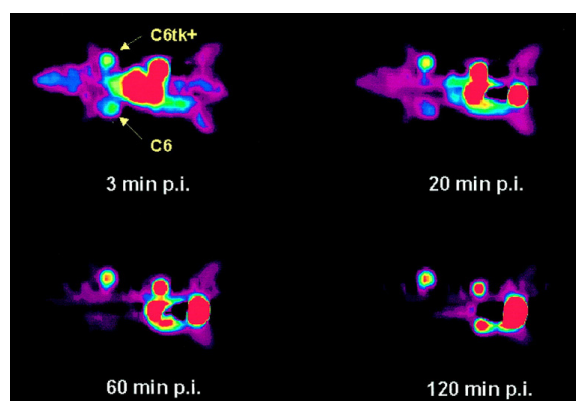


Figure 4 PET imaging of herpes simplex virus-thymidine kinase (HSV-Tk) gene transfection into a murine tumour. The C6tk+ tumour cells were transfected with HSV-Tk and implanted into nude mice. Control tumours had C6 cells only. HSV-Tk causes phosphorylation and intracellular trapping of the radiotracer 9-[(1-[^{18}F]Fluoro-3-hydroxy-2-propoxy)methyl]guanine ([^{18}F]FHPG). Note persistence of activity in the C6tk+ tumour indicating gene transfection. Reproduced with permission^[89].

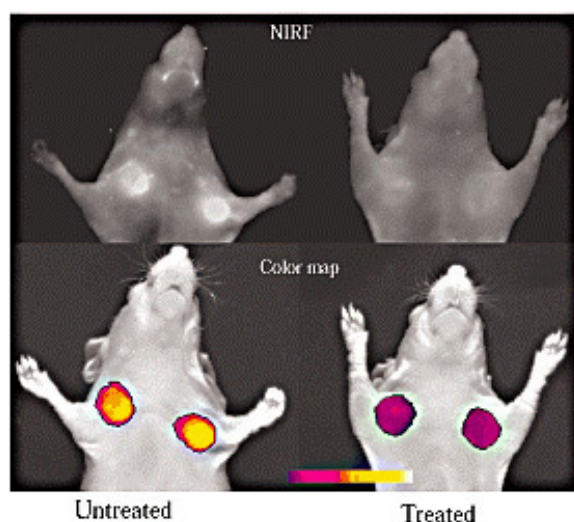


Figure 5 Near infrared (NIR) imaging of the inhibition of the enzyme matrix metalloproteinase-2 (MMP-2) in a murine tumour model using a 'smart' fluorescent probe. In the absence of the MMP inhibitor the NIR probe binds to its MMP target and is converted to a fluorescent substrate. The smart NIR probe will not be activated without binding to the enzyme. Reproduced with permission^[58]. Copyright Nature Publishing Group.

PET

PET is the current paradigm for sensitivity to molecular events with *in vivo* imaging^[39]. Animal systems are now available providing 1–2 mm in-plane resolution^[10]. PET (and other radionuclide techniques) is ideally suited to imaging molecular events because of its sensitivity to biologically relevant quantities of compound (nano to femtomolar). The technique is limited by

access to hardware and cyclotrons (the half-life of many radionuclides is in the order of minutes). Most importantly PET is quantitative^[40] and provides the ability to characterise receptors and ligands at molecular concentrations^[41]. PET is extensively used in the development of psychiatric^[42,43] and anticancer^[44] drugs. Isotopic compound labelling is a great strength in drug development. A putative therapeutic compound labelled with a Gd(III) would be chemically unrecognisable to its parent compound. Examples of PET radionuclides used for MI include ^{15}O (2.07 min), ^{13}N (10 min), ^{11}C (20.3 min), ^{18}F (1.83 h), ^{124}I (4.2 days), and $^{94\text{m}}\text{Tc}$ (53 min).

PET radionuclides based on ^{18}F such as ^{18}F -flurothymidine (a marker for tumour proliferation) and ^{18}F -fluorodeoxyglucose (a glycolysis marker) have been particularly useful in probing neoplastic processes and are entering clinical research^[45,46]. Strictly speaking these are not specific molecular markers as they report on global cellular processes. Preclinically PET has been particularly useful in monitoring extracellular receptor expression and the efficacy of gene therapy vectors^[47,48]. Arguably, the best example of gene transfer imaging is the herpes simplex virus-thymidine kinase (HSV-Tk) system^[49]. Thymidine kinase catalyses substrate phosphorylation, leading to the phosphorylated substrate being 'trapped' intracellularly (Fig. 4). If a substrate has an ^{18}F substitution it is acceptable to infer that HSV-Tk has been incorporated into the host genome by the persistence of radioactive counts over the region of interest. The imaging of transcriptional regulation^[50,51] elegantly illustrates the sensitivity of PET to a central cellular process. Transcription (the process of transcribing DNA to mRNA) is a core mechanism in the cellular manufacture of proteins and is a common focal point of cellular disruption in cancer.

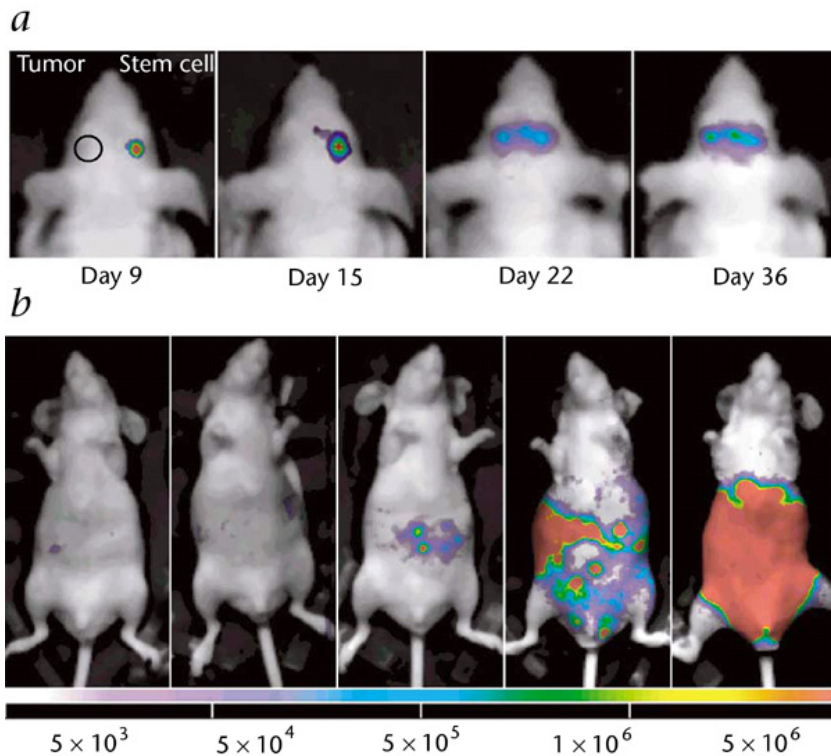


Figure 6 Examples of bioluminescence imaging. (a) Migration of neural progenitor stem cells across the midline towards an implanted glioma in a mouse. The neural progenitor cells were labelled with the luciferin (*luc*) gene *ex vivo* so that cells expressing luciferin exhibit luminescence under appropriate conditions. (b) *luc*-labelled ovarian cancer cells implanted at different densities in the peritoneal cavity of a nude mouse. Note that cellular densities as low as 5×10^3 cells can be imaged. Reproduced with permission^[53]. Copyright Nature Publishing Group.

Single photon emission computerised tomography (SPECT)

SPECT is a powerful technique that has been used to image several molecular processes at a fraction of the cost of PET. SPECT detects γ -emitting radionuclides by rotating a photon detector array around the body thereby acquiring multiple projections. Sodium iodide or solid-state cadmium-zinc-telluride can be used to detect emitted γ -photons with a spatial resolution of 1–2 mm. Typical SPECT radionuclides include ^{99m}Tc (6 h), ^{111}In (2.8 days), ^{123}I (13.2 h) and ^{125}I (59.5 days). SPECT has commonly been used to track molecules and cells including the radiolabelled annexin-V as an early marker of apoptosis^[52].

Optical imaging

Optically-based methodologies are having a substantial impact on MI^[53]. Compared with other imaging strategies optical imaging is cheap, has a spatial resolution of 1–2 mm (1 μm for intravital microscopy) and possesses

nanomolar sensitivity. Its principal limitation is depth penetration but recent simulations suggest that tissue penetration could reach several centimetres in depth (see below).

Optical imaging technology has been enabled because of the modelling of tissue light propagation, the development of biocompatible near infrared (NIR) probes^[54,55] and the development of sensitive photon detection technologies^[56,57]. Several optical processes can be exploited but fluorescence and luminescence primarily support studies *in vivo*. Fluorescence is the absorption of light at one wavelength (thereby requiring a light source) and its emission at a lower wavelength. Luminescence on the other hand does not require a light source and arises by the conversion of chemical energy to light. Generically bioluminescence (that is luminescence occurring in an organism) is typified by the luciferin/luciferase reaction where the former is the substrate and the latter the catalysing enzyme (the reaction requires ATP and O_2). The male firefly *Photinus pyralis* exploits this reaction when emitting its characteristic flashes of light. An excellent review of bioluminescence can be found at <http://www.lifesci.ucsb.edu/~biolum/>.



Figure 7 Cartoon depicting the molecular structure of green fluorescent protein (GFP). GFP consists of an 11-stranded β -barrel in addition to a central helix that carries the chromophore. GFP therefore acts as an energy acceptor for the protein aequorin efficiently transforming blue light (470 nm) emitted by aequorin into green light (508 nm). Structure downloaded from Protein Data Bank (1EMA): <http://www.rcsb.org/pdb/index.html>.

Fortunately for *in vivo* optical imaging the lowest coefficient of absorption (haemoglobin is the principal absorber of visible light whilst water and lipids principally absorb infrared) *in vivo* occurs at 650–900 nm. This range of wavelengths coincides with minimal autofluorescence (this is the inherent fluorescence of tissues). The combination of photochemistry and the ‘smart’ probe concept has stimulated the synthesis of NIR fluorochromes and reporter probes. These substances become activated in the presence of a molecular target, which amplifies the emitted light. Various receptor substrates have been synthesised including folate, tumour cell and protease receptor targeted probes. In the therapeutic arena ‘smart’ NIR fluorochromes have probed and demonstrated the pharmacodynamics of matrix metalloproteinase-2 inhibition (MMP-2) as early as 8 h (Fig. 5) following initiation of therapy^[58]. The greatest challenge to optical imaging technology will be to translate it to opaque animals. Fluorescence molecular tomography (FMT) may provide a solution. In FMT an object is rotated within an array of emitter/receiver charged couple devices. The resulting spatially encoded fluorescence is reconstructed tomographically. The result is a quantitative 3D map with nanomolar sensitivity and spatial resolution of 1–2 mm. Recent modelling^[59] suggests that 7–14 cm of tissue penetration is achievable using appropriate fluorochromes.

The use of fluorescent proteins and bioluminescence in MI merits comment. Both approaches are commonly used preclinically to track cells including stem and tumour cells (Fig. 6) and as gene reporters^[60,61]. Green fluorescent protein (GFP) (Fig. 7) was originally extracted from the jellyfish (Fig. 8) *Aequorea victoria*^[62] (information on *Aequorea* bioluminescence

can be found at <http://faculty.washington.edu/cemills/Aequorea.html>). The use of GFP suffers because its emission wavelength (510 nm) is close to the autofluorescence of many tissues. Spectrally ‘red-shifting’ the protein via genetic engineering of GFP has led to the development of red fluorescent protein (RFP). RFP can then be imaged quantitatively at greater depths than GFP. One advantage of bioluminescence is there is no inherent background which optimises photon statistics. The technique is, however, semi-quantitative (it provides a yes/no paradigm).



Figure 8 The bioluminescent protein GFP was originally extracted from the jellyfish *Aequorea victoria*. Although many examples of bioluminescence exist in nature the image does not represent bioluminescence. The jellyfish was illuminated by a lamp during photography. Reproduced with permission of the author.

Translating MI to man—the MR approach in oncology

Probing tumour vascularity

Clinical imaging is increasingly called upon to study molecular-based phenomena in man. The techniques of permeability (capillary leakiness), perfusion and diffusion weighted imaging (DWI)^[63–66] all feature

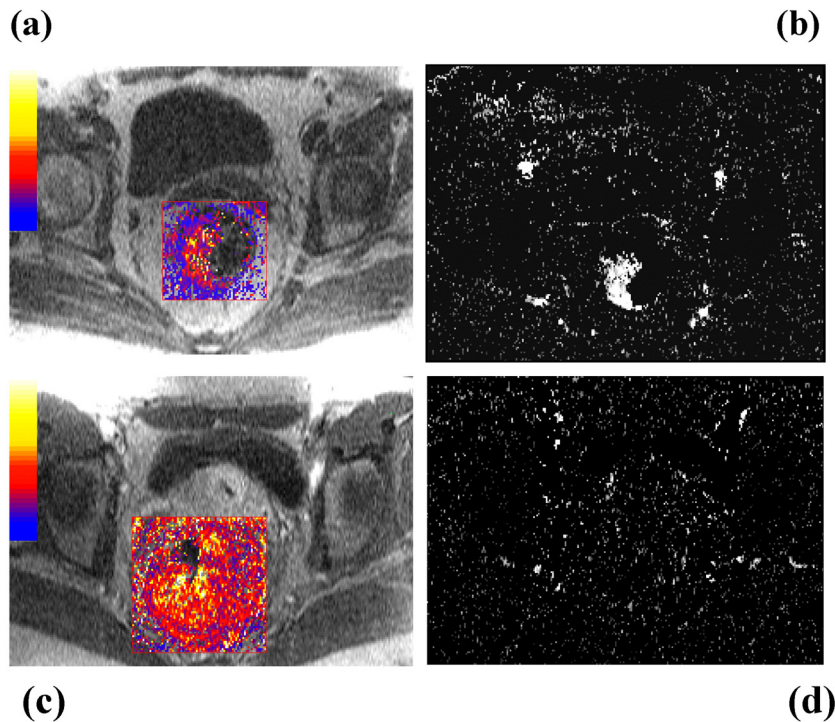


Figure 9 Parametric maps from a permeability (a, c) and perfusion (b, d) in two rectal tumours (a, b; c, d). The degree of permeability in tumours ‘a’ and ‘c’ can be gauged from the colour scale. The relative blood volume maps for the two tumours are given in ‘b’ and ‘d’. The permeability maps are derived by fitting gadolinium concentration data to a kinetic model and are based on mapping T1. The relative blood volume maps are derived from first pass T2* susceptibility data. Evidence suggests that tumours that have high permeability and perfusion measured via this technique are more likely to respond to chemotherapy than those that do not.

prominently. Permeability (or more strictly capillary leakiness) and perfusion are determined by the analysis of dynamically acquired paramagnetic contrast agent data (i.e. Gd-DTPA, gadolinium diethylenetriamine pentaacetic acid). The data are analysed by fitting the MR output to a kinetic model^[67]. Several kinetic models are in use and consensus is gradually becoming established^[68]. The relationship of these techniques to the underlying biology, however, remains to be more widely validated^[67]. Essentially, T1 data are recorded using a ‘fast’ sequence, typically a gradient-echo at repeated (seconds) intervals over a predetermined time (5–7 min). As a result of the administration of the contrast agent the T1-weighted signal in the region of interest will be modulated and recorded over the time course of the study. With appropriate processing a serial set of T1 maps is created to which a given model is fit. The result is a pixel-by-pixel map of the contrast agent concentration. Other model parameters include capillary leakiness (K^{trans}) and extracellular volume (v_e). Estimates of perfusion depend on recording the susceptibility (T2*) effect of a paramagnetic contrast agent on its first pass through a designated region of interest. The resultant drop in signal intensity below noise is then related to the blood volume in that region.

In locally advanced rectal cancers (Fig. 9) permeability determined preoperatively was associated with response to chemotherapy^[69].

Application in clinical trials

Several new therapeutic strategies^[70,71] are targeting pathologic vascularity and angiogenesis^[72]. Some of these agents are currently completing phase I trials. MR studies supporting these phase I trials have demonstrated data supporting MR as a pharmacodynamic endpoint^[73]. No doubt such imaging studies will increasingly feature in clinical trials.

DWI of tumours

Diffusion is the random motion of molecules down a concentration gradient. The process is rapid in gases, slow in solids and intermediate in liquids. Fortunately the translational motion of molecules in liquids is within the sensitivity range of MR. MR can quantitatively define diffusion such that an image can be assigned a numerical diffusion value on a pixel-by-pixel basis. Under controlled conditions water returns a unique value

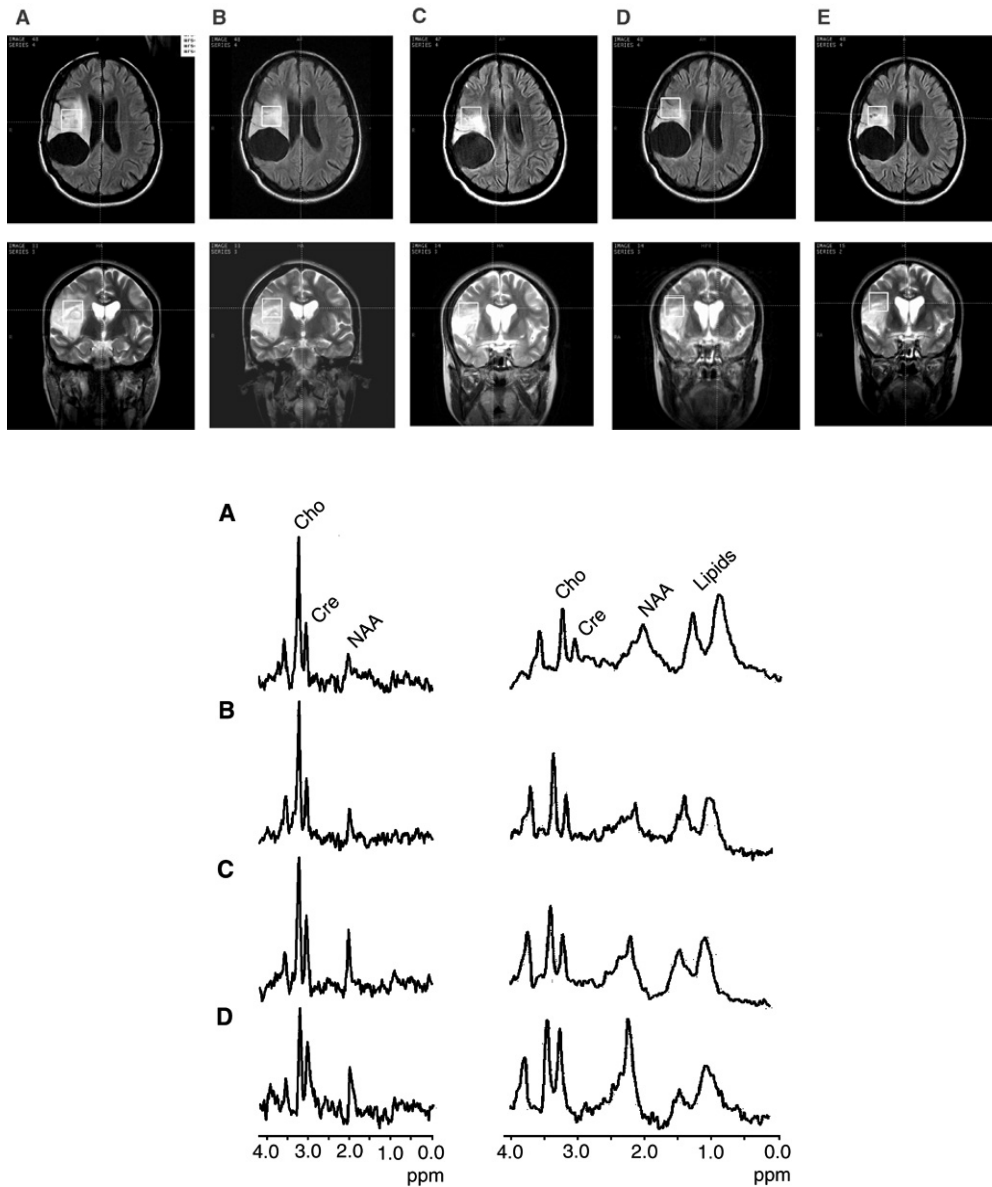


Figure 10 ¹H-MRS can be used to follow metabolic tumour changes during chemotherapy in man^[90]. The axial short tau inversion recovery (STIR) (top) images and coronal (bottom) images demonstrate the size change in this low-grade glioma receiving temozolomide therapy. Note the posteriorly sited post-surgical cavity. Note the diminishment in the ratio of choline (Cho) to creatine (Cre) from spectra A to D. The spectral changes paralleled size change in tumours. The study suggests that it may be possible to monitor treatment-induced changes non-invasively. Copyright Nature Publishing Group.

for diffusion termed the diffusion constant ($\text{mm}^2 \text{s}^{-1}$). A glass of water consists of only one compartment whilst *in vivo* a tissue holds several compartments. Each of these can be defined by a unique diffusion value and therefore it is inappropriate to think of an *in vivo* diffusion constant. The ‘apparent diffusion coefficient’ (ADC) is therefore used as a summary value of the various diffusion weightings of tissues. Alternatively ADC can be thought of as a measure of cellularity. Necrosis at one extreme presents a limited barrier to the motion of water molecules. Highly cellular environments on the other

hand are considerably more restrictive to the diffusion of, for example, water molecules. By this premise ADC in locally advanced rectal tumours was found to be strongly correlated with response to chemotherapy and chemoradiation^[74]. On the basis of preclinical results necrosis is likely to be the physiological factor underlying this association^[75,76]. In animal model tumours DWI has convincingly demonstrated changes in tumour ADC prior to change in tumour size^[76–80]. There is therefore considerable precedent to translate this technology into man (Fig. 10).

MRS

Without a nuclear magnetic moment, MR as a discipline would be non-existent. However, if a nucleus only resonated at one frequency it would be impossible to distinguish one molecule from another and MR would not be the powerful analytical technique that it is. The existence of unique resonances arises via the interaction of the magnetic field generated by orbital electrons and the main magnetic field B_0 . The preferential shielding of one nucleus over another results in unique spectroscopic signatures. Although the technique is limited in sensitivity, particularly at clinical field strengths, it permits the interrogation of tissue biology (Fig. 10) and/or drug metabolism non-invasively in man^[81]. ^{19}F -MRS (where the recorded MR signal is from the ^{19}F nucleus) in particular has been applied to studies of anticancer^[82,83], antibiotic^[84] and anaesthetic^[85,86] drug metabolism. Furthermore, ^{19}F -MRS has been used to predict the response of treatment to 5-fluorouracil (5FU)^[87] and has demonstrated the biliary excretion of 5FU catabolites in man^[88]. The use of stronger B_0 fields in man is likely to promote the use of MRS in the study of tumour and drug metabolism.

Conclusion

MI is an established technique for the imaging of biologic events *in vitro*. By combining molecular biology, chemistry and imaging technologies MI is beginning to impact substantially on preclinical *in vivo* studies. The advent of molecular anticancer treatment strategies should be the catalyst that translates MI to the clinic.

Declared interests and acknowledgements

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