



Molecular Imaging of Cancer

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Patients with similar tumour types frequently have markedly different responses to the same therapy. The development of new treatments would benefit significantly, therefore, from the introduction of imaging methods that allow an early assessment of treatment response in individual patients, allowing rapid selection of the most effective treatment (Brindle, *Nat. Rev. Cancer* 2008; 8:1).

Introduction

Tumour responses to treatment are still largely assessed from imaging measurements of reductions in tumour size. However, this may take several weeks to become manifest and in some cases may not occur at all, despite a positive response to treatment. We have been developing non-invasive and clinically applicable magnetic resonance-based methods for detecting the early responses of tumours to therapy. A primary focus has been on the development of methods for detecting tumour cell apoptosis, or programmed cell death, since the level of tumour apoptosis after drug treatment has been shown to be a good predictive indicator for treatment outcome in preclinical and clinical studies. Thus by monitoring tumour cell death an oncologist may get an indication of whether a particular drug is working very early during treatment, possibly within 24-48 hours, and long before there is any evidence of tumour shrinkage.

A targeted imaging agent for detecting cell death

A major focus has been the development of a targeted contrast agent that allows detection of apoptotic cells by MRI. We showed previously that the C2A domain of the protein synaptotagmin would bind to the phosphatidylserine (PS) that appears on the surface of apoptotic cells. We have recently developed Gd³⁺-based versions of this agent, which give positive contrast and are therefore easier to detect than earlier versions, and which are smaller and thus more readily enter the tumour interstitium (Krishnan et al., *Radiology* 2008; 246:854). While these MR agents are very powerful for preclinical imaging their translation to the clinic remains a significant challenge. Therefore we are developing an agent that could be used for radionuclide imaging (PET and SPECT) and which could be translated into clinical application. We have made a site-directed mutant of C2A (C2Am) in which we have replaced a serine residue in a loop region distant from the active site with a cysteine residue (S78C). The mutant has an affinity for PS that is indistinguishable from the wild-type protein and, using sulphhydryl-selective agents, we have been able to produce homogeneous preparations of C2Am, with just a single label present. The agent appears to be superior to the current 'gold standard' for PS-detection, annexin V, in that it shows less non-specific binding to viable cells. A patent on this agent has been filed.

Imaging metabolism with hyperpolarized ¹³C-labelled cell substrates

There are several metabolic markers of cell death that can be detected using ³¹P and ¹H magnetic resonance spectroscopy. However, the problem with these MRS measurements is that they are relatively insensitive, and therefore lack both temporal and spatial resolution, which has limited their

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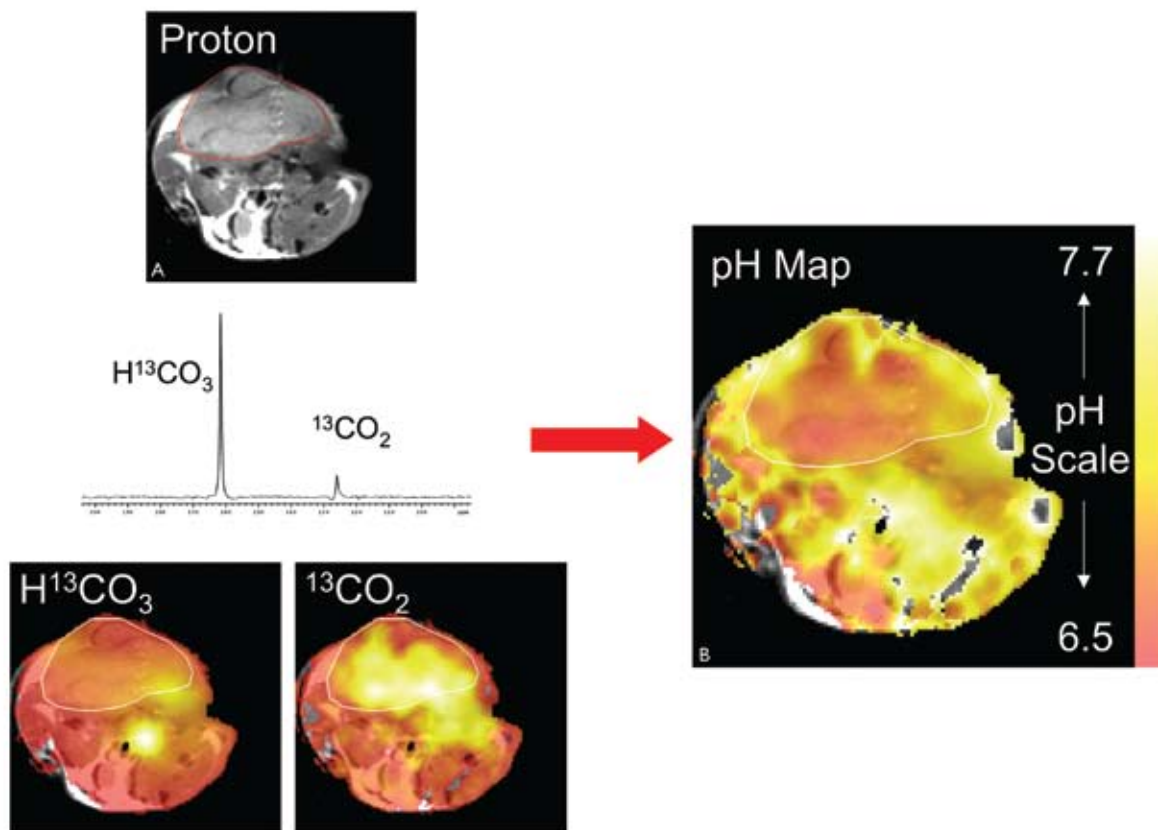


Figure 1. (a) Transverse proton magnetic resonance image of a mouse with a subcutaneously implanted EL4 tumour (outlined in red). (b) pH map of the same animal calculated from the ratio of the $H^{13}CO_3^-$ and $^{13}CO_2$ voxel intensities in ^{13}C chemical shift images acquired ~ 10 s after intravenous injection of ~ 100 mM hyperpolarized $H^{13}CO_3^-$ and assuming a pK_a of 6.17. The tumour margin is outlined in white.

application in the clinic. Sensitivity in the NMR experiment can be increased dramatically, however, using nuclear spin hyperpolarization techniques. We have been developing this novel and exciting technology in collaboration with GE Healthcare. We have shown that exchange of hyperpolarized ^{13}C label between the carboxyl groups of lactate and pyruvate, in the reaction catalyzed by the enzyme lactate dehydrogenase, can be imaged in tumours and that this flux decreases in treated tumours undergoing drug-induced cell death (Day et al., *Nat. Med.* 2007; 13:1382). We have shown recently that although the PET measurements of [^{18}F]-fluorodeoxyglucose (FDG) uptake detect an earlier treatment response in lymphoma tumours than the polarized pyruvate experiment, the decrease in the rates of FDG uptake and lactate-pyruvate exchange were comparable (submitted for publication). A clinical trial of the polarized pyruvate experiment is planned for next year. We have also shown that we can monitor the conversion of hyperpolarized [$5-^{13}C$]glutamine to glutamate in human hepatoma cells *in vitro*. Since glutaminase activity has been correlated with the rate of cellular proliferation, measurements of its activity *in vivo* could be used to detect the early responses of

tumours to cytotoxic and cytostatic drugs (Gallagher et al., *Magn. Reson. Med.* 2008; 60:253).

Alterations in tissue pH underlie many pathological processes, and therefore the capability to image tissue pH in the clinic could offer new ways of detecting disease and response to treatment. We have shown that tissue pH can be imaged *in vivo* from the ratio of the signal intensities of hyperpolarized $H^{13}CO_3^-$ and $^{13}CO_2$ following intravenous injection of hyperpolarized $H^{13}CO_3^-$ (Figure 1) (Gallagher et al., *Nature* 2008; 453:940).

Future directions

We are investigating a novel approach for imaging cell surface glycans which involves feeding modified azido-sugars and detecting the resulting cell surface glycans *in vivo* using Cu-free click chemistry and radionuclide-labelled SPECT and PET probes. We are also investigating the potential of ^{11}C -acetate as a PET agent for cell death detection.

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