



Molecular Imaging of Tumour Cell Death

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The goal of the Molecular Imaging Laboratory is to develop magnetic resonance methods for the evaluation and design of novel tumour therapies, including immunotherapy and anti-vascular and gene therapies.

A better understanding of the molecular signature of cancer has allowed the development of a new generation of anti-cancer drugs that target specific molecular entities, such as receptors, genes, or signalling pathways. However, DNA microarray-based disease profiling, together with the results of recent clinical trials using targeted therapies, have clearly demonstrated the intrinsic heterogeneity of human tumours, both genetically and phenotypically. Patients with similar tumour types frequently have markedly different responses to the same therapy. The development of these novel targeted cancer therapies could benefit significantly, therefore, from the introduction of imaging methods that allow an early assessment of treatment response in individual patients. These would allow an oncologist to rapidly assess the effectiveness of a new therapy. Ineffective treatments could be abandoned at an early stage and more effective treatments selected, with attendant welfare benefits for the patient and cost benefits for the health care system (Neves and Brindle, *Biochim. Biophys. Acta* 2006; 1766:242; Brindle, *Nat. Rev. Cancer* 2008; In press).

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, in preclinical and clinical studies, to be a good prognostic indicator for treatment outcome. 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 major focus has been the development of novel targeted contrast agents that allow detection of apoptotic cells by MRI. We showed that the C2A domain of the protein synaptotagmin would bind to the phosphatidylserine that appears on the surface of apoptotic cells. When conjugated to small paramagnetic iron oxide (SPIO) particles this protein allowed MRI detection of apoptotic cells, both *in vitro* and *in vivo*, in a tumour treated with chemotherapeutic drugs (Zhao *et al.*, *Nat. Med.* 2001; 7:1241). More recently we have developed Gd³⁺-based versions of this agent, which give positive contrast and are therefore easier to detect, and which are smaller and thus more readily enter the tumour interstitium (Jung *et al.*, *Bioconjug. Chem.* 2004; 15:983; Neves *et al.*, *Nano Lett.* 2007; 7:1419). A paper describing the first *in vivo* application of one of these Gd³⁺-based agents is currently in press (Krishnan *et al.*, *Radiology* 2008; In press).

Sensitivity in the NMR experiment can be increased

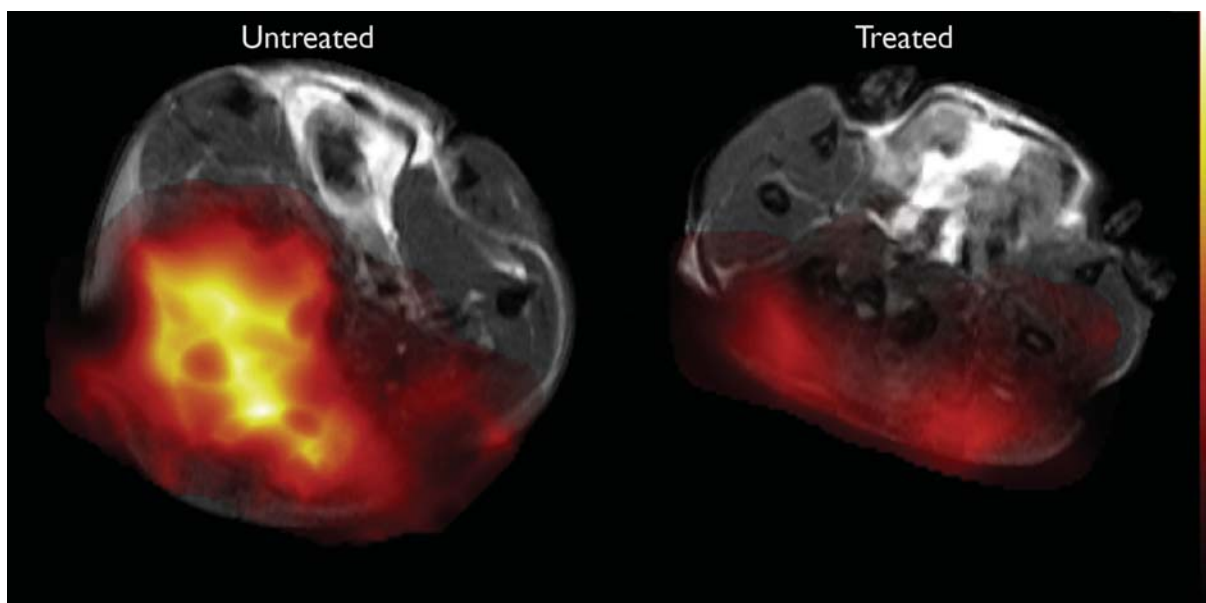


Figure 1. Images of tumours before and after drug treatment. The grey-scale image shows tissue anatomy. The false colour image indicates the activity of lactate dehydrogenase in the tumour, which is decreased in dying cells (the less yellow the colour the lower the enzyme activity).

dramatically using nuclear spin hyperpolarization techniques. The conventional way to increase nuclear spin polarization, and thus the sensitivity of the NMR experiment, is to use a higher magnetic field. An alternative way is to transfer polarization from an electron or nuclear spin that has a higher polarization. A versatile technique, which in principle can be used with any NMR-active nucleus, involves transferring polarization from a hyperpolarized electron spin (Ardenkjaer-Larsen *et al.*, *PNAS* 2003; 100:10158). In practice it means using nuclei that have relatively long T_1 relaxation times, for which the lifetime of the polarization is sufficiently long-lived (typically tens of seconds) to be detected in a subsequent imaging experiment. In a dynamic nuclear polarization (DNP) experiment a solution of the material to be polarized is mixed with a stable radical and rapidly frozen to form a glass. The electron spin on the radical is polarized (to unity polarization) by cooling the sample to a very low temperature (~ 1.3 °K) in a magnetic field. The polarization is then transferred to the nuclear spin by exciting the electron spin resonance and currently nuclear spin polarizations of up to 50% are possible in favourable cases. The polarized compound is then brought rapidly to room temperature, without significant loss of this polarization, and injected into the biological system (Golman *et al.*, *PNAS* 2003; 100:10435; Golman *et al.*, *PNAS* 2006; 103:11270; Golman and Petersson, *Acad. Radiol.* 2006; 13:932; Golman *et al.*, *Cancer Res.* 2006; 66:10855). Since detection of the polarization necessarily involves its destruction, the imaging experiments that are used must make efficient use of this enhanced polarization.

We showed recently that exchange of a hyperpolarized ^{13}C label between the carboxyl groups of lactate and pyruvate, in the reaction catalyzed by the enzyme lactate dehydrogenase,

could be imaged in tumours and that this flux was decreased in treated tumours undergoing drug-induced cell death (Day *et al.*, *Nat. Med.* 2007; 13:1382)(Figure 1). We have suggested that this technique could be used in the future for response monitoring in the clinic, in much the same way as ^{18}F FDG has been used with PET. We have also shown that we can monitor the conversion of hyperpolarized $[5-^{13}\text{C}]$ 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, as has been done with 3'-deoxy-3'- $[^{18}\text{F}]$ fluorothymidine (FLT) and PET.

Since alterations in tissue pH underlie many pathological processes, the capability to image tissue pH in the clinic could offer new ways of detecting disease and response to treatment. We have shown very recently that tissue pH can be imaged *in vivo* from the ratio of the signal intensities of hyperpolarized $\text{H}^{13}\text{CO}_3^-$ and $^{13}\text{CO}_2$ following intravenous injection of hyperpolarized $\text{H}^{13}\text{CO}_3^-$. The technique was demonstrated with a study on a mouse tumour model, which showed that the average tumour pH was significantly lower than the surrounding tissue. Since bicarbonate is already used intravenously in humans, we propose that this technique could be used clinically to image tumours and other pathological processes that are associated with alterations in tissue pH such as ischemia and inflammation.

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