Cellular MRI for Mapping Proliferation During Tumour Development

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Introduction: Tumours are comprised of a diverse population of cells, only a small subset of which is able to initiate and propagate disease.[1] These cells, referred to as cancer stem cells (CSC), act as seed points for tumour proliferation, producing diverse progeny in the process, that ultimately account for the cellular makeup of the tumour.[2] Simple tumour volume measurements, the most common evaluation of tumour progression and treatment response in cancer models, fail to account for this functional and cellular heterogeneity. Improved progression metrics, including a spatial map of proliferative history, would provide better evaluations of emerging cancer treatments. Cellular MRI techniques, using intracellular iron oxide contrast agents, allow labelled cells to be tracked *in vivo* over time[3-4], but the contrast agent is diluted by cell division; a phenomenon that may allow proliferative activity within a population of labelled cells to be spatially mapped longitudinally.[5] If so, longitudinal cellular MRI of tumour growth would provide a way of characterizing the CSC population of a tumour or cell line and provide improved monitoring of response to treatment.

Hypothesis: In this work, we seek to test the hypothesis that dilution of iron oxide in a growing tumour can be used to represent a spatial map of proliferative activity in the GL261 murine glioma model.

Methods: GL261 cells were labelled with micron-sized iron oxide particles (MPIO) in culture and then injected into the brains of C57Bl/6 mice and allowed to develop into tumours. The mice were imaged *in vivo* on a 7T MRI scanner using a T2-weighted 3D-fast spin echo (FSE) protocol that was optimized for tumour visualization and conspicuity of variation in iron concentration through phantom experiments (TR=1800ms, echo spacing=10ms, TE_{eff}=40ms, ETL=12, 125µm isotropic resolution). Perls Prussian Blue (PPB) staining of brain sections was performed to confirm that hypointense contrast on MR images was due to MPIO. To validate that the observed iron distribution also mapped cell proliferation—and not decreased cell motility or MPIO expulsion—the cells were co-labelled with Cell Tracker Orange (CTO), a fluorescent dye used for optical studies of cell proliferation and known to be well-retained and divided equally between daughter cells. Optical projection tomography (OPT) was used to obtain 3D *ex vivo* optical images of the distribution of CTO within the tumours, which were registered to the MR images to allow direct comparison. Vibratome tissue sections were also stained for PPB in order to compare the location of CTO and iron using standard microscopy.

Results & Discussion: Longitudinal MRI of labelled tumour growth showed regions of high MR signal intensity, suggestive of cells having undergone several divisions, mainly in the tumour periphery, with the tumour core remaining hypointense until the latest, terminal tumour stages (Fig. 1). PPB staining confirmed that the contrast on the MR images reflected a "core-centred" iron distribution. The distribution of CTO-labelled cells observed on OPT images also confirmed the MR image data, and comparison with PPB-stained sections showed many CTO-labelled cells colocalized with iron (Fig. 2). Together, these results suggest that the distribution of iron observed on *in vivo* MR images is a marker of cell proliferation consistent with histological markers, and that cell proliferation/migration was not hindered by iron labelling.

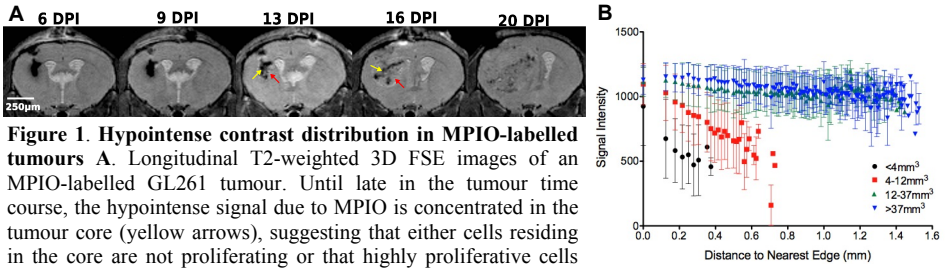

Figure 1. Hypointense contrast distribution in MPIO-labelled tumours A. Longitudinal T2-weighted 3D FSE images of an MPIO-labelled GL261 tumour. Until late in the tumour time course, the hypointense signal due to MPIO is concentrated in the tumour core (yellow arrows), suggesting that either cells residing in the core are not proliferating or that highly proliferative cells tend to relocate to the tumour periphery (red arrows). **B**. Plot showing quantitative confirmation of observed growth pattern. Average normalized signal intensity from 5 tumours at different stages of development is plotted as a function of distance from the tumour edge. (DPI=days post injection; error bars represent standard deviation)

Conclusion: We confirm here the hypothesis that iron labelling of tumour cells can be used to map proliferative regions within a developing tumour. In further investigations, we will manipulate the tumour growth dynamics by modifying the injected cell population in order to study the factors that contribute to the growth patterns and to verify that CSC content is reflected functionally in a proliferation map. With targeted therapies being the focus of novel treatments, these methods will provide a new platform for preclinical evaluation of cancer therapies.

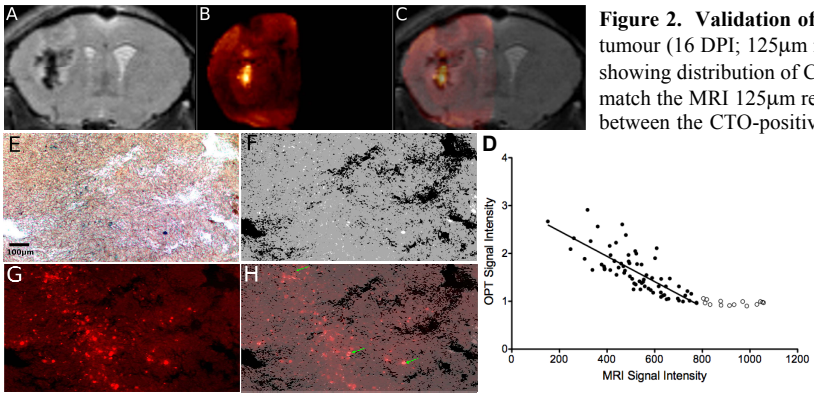

Figure 2. Validation of MRI data. A. MR image through tumour of MPIO- and CTO-labelled tumour (16 DPI; 125µm resolution). **B.** Fluorescence OPT image of half of the brain shown in A, showing distribution of CTO within the tumour. Image shown has been resampled from 7.4µm to match the MRI 125µm resolution. **C.** Overlay of images in A and B showing excellent agreement between the CTO-positive region and the area of low signal intensity on MRI. **D.** Plot showing quantitative confirmation of the correlation between normalized OPT and MRI signal intensities from a voxel-by-voxel comparison of the registered OPT and MRI images shown in A-B. Open circles indicate voxels that show dilution of the contrast agents and were therefore excluded from the regression analysis. For voxels retaining the agents, the OPT and MRI data agreed very well (R^2=0.6168, p<0.0001). **E.** Brightfield microscopy image of PPB-stained 50µm vibratome section from the same tumour. Blue colour indicates the presence of iron. **F.** Image from E classified into iron-positive (white), counterstain (grey), and background (black). **G.** Fluorescence microscopy image of the same section seen in E & F showing distribution of CTO. **H.** Overlay of classified image in F with fluorescent image in G showing excellent correspondence between PPB and CTO labelling. Green arrows indicate examples of cells positive for both iron and CTO.

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References: [1] Singh et al., Oncogene 23:43, 7267-73 (2004) [2] Soltysova et al., Neoplasma 52:6, 435-40 (2005) [3] Nieman et al., Neuroimage 50:2, 456-64 (2010) [4] Heyn et al., Magn Reson Med 55:1, 23-9 (2006) [5] Magnitsky et al., ISMRM, 2010