Visualizing Invading Glioma Cells with MRI at the Single Cell Level

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Introduction

Gliomas are the most common primary tumours of the central nervous system and often present an extremely poor prognosis that has been attributed to the extensive invasion of tumour cells into the surrounding brain tissue. Single cells invade actively along white matter tracts and vascular basal laminae, making complete surgical resection impossible and limiting the success of radiation and chemotherapy. Invading cells are also responsible for disease recurrence as well as for progressive neurological dysfunction.

The *in vivo* assessment of glioma cell invasion has been limited by the static picture provided by histological studies. Histological evaluation is a time-consuming and labour-intensive process that yields only a single measurement per experimental animal. [1] The ability to observe cell migration dynamically would greatly improve on the efficiency of *in vivo* experiments and would allow for better assessment of the invasion process and responses to treatment.

Several groups have shown that the intracellular incorporation of superparamagnetic iron oxide (SPIO) contrast agents enables the visualization of single cells *in vitro* with MRI, but most of these studies have been done at field strengths exceeding 4.7 T. [2,3,4] *In vivo* imaging of SPIO-loaded cells typically requires that large numbers of cells be transplanted into the tissue, and the limit of detection has been small groups of cells. [5] We have previously shown that single SPIO-loaded cells can be detected *in vitro* at 1.5 T using a customized micro-imaging protocol that uses a gradient insert coil and the 3DFIESTA pulse sequence. [6]

The underlying hypothesis for this work is that single invading glioma cells can be observed and studied *in vivo* using MRI. The objective of the current work was to develop the necessary tools to test this hypothesis in a three-dimensional model of cell invasion. We show that the progression of glioma cell invasion through a collagen matrix can be observed over several days using our micro-imaging system at 1.5 T.

Methods

Sample Preparation: The rat C6 glioma cell line was chosen for its excellent homology to human gliomas, including cell invasion. Cells were labelled with micron-sized superparamagnetic and fluorescent polystyrene microbeads (mean diameter 0.9µm, Bangs Laboratories, Inc.) by incubating them with different microbead concentrations for 24 hours. Cells were washed of excess beads, trypsinized, and then grown on 1.5% agar-coated plates and allowed to form multi-cellular tumour spheroids (MTS) for several days. Single spheroids (200-300µm diameter) were transferred into 350µl microwells containing collagen type I gel (Vitrogen 100, Cohesion Technologies), a matrix through which the cells invade, and observed over several days using MRI and phase contrast microscopy.

MR and optical imaging: MR imaging was conducted on a 1.5 T clinical scanner (GE) with the addition of a custom-built gradient insert coil (gradient strength 600mT/m and slew rate 2000T/m/s), enabling high-resolution images in reasonable scan times. The fully balanced SSFP sequence 3DFIESTA (Fast Imaging Employing Steady State Acquisition) was used because of its high sensitivity to iron (TR/TE 7.1/3.6ms, flip angle 30°, BW 20.83kHz, scan time ~ 12mins for 4 NEX at 100µm isotropic resolution). Phase contrast microscopy was conducted on an Olympus IX50 inverted microscope equipped with a Sony 3CCD colour digital camera.

Results

Bead Uptake and Single Cell Detection: Cellular iron content, Fe_{cell}, was measured using a susceptometry technique—the Reilly-McConnell-Meisenheimer (RMM) method—modified to use multi-echo imaging and was found to increase with the iron incubation concentration, [Fe], with the form $Fe_{cell} = C(1-e^{-[Fe]/k})$. Cellular incorporation of microbeads did not affect cell proliferation or plating efficiency and the iron was distributed evenly upon cell division without being expelled or degraded by the cells (data not shown). Iron-loaded cells appeared as areas of signal void on MR images and single cell detection was confirmed through correlation with phase contrast microscopy (Figure 1).

Multi-cellular tumour spheroids (MTS): Iron-loaded rat C6 glioma cells formed MTS when grown on plates base-coated with 1.5% agar (Figure 2). When imbedded in collagen type I gel, cells on the outer rim of the MTS began invading the gel within the first few hours, and by the next day, single cells were observable under the microscope up to 200µm from the original implantation site. The corresponding MR images showed mild invasion on the second day, but by a week later, extensive invasion was observable on both the MR and phase contrast images (Figure 3).

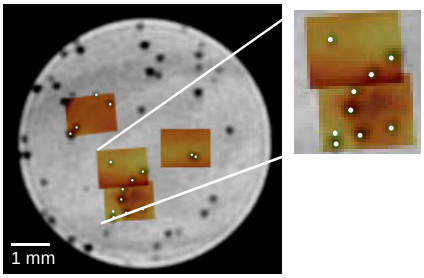

Figure 1. MR image of rat C6 glioma cell monolayer overlaid with corresponding phase contrast images. Areas of signal void on the MR image correspond to one or two cells (indicated by the white dots). This confirms that rat C6 glioma cells incorporate microbeads to a sufficient extent to allow detection of single cells *in vitro*.

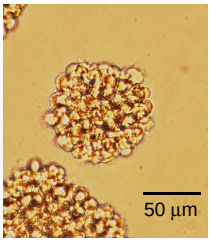

Figure 2. Rat C6 glioma MTS growing on 1.5% agar 48 hours after plating. Typically, MTS were harvested and imbedded in collagen gel at 96 hours, when they measured 200-300µm in diameter.

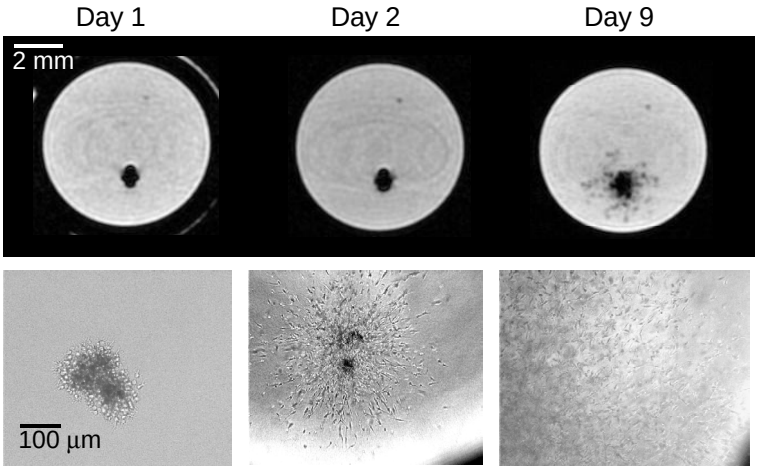

Figure 3. MR images (top row) and corresponding phase contrast images (bottom row) of rat C6 glioma MTS imbedded in collagen type I gel on the day of implantation, the next day, and a week later. On day 1, the MTS is a tight group of cells with an outer rim that has just begun to invade the gel. By day 2, single cells have invaded the gel up to 200µm, as observable on the middle phase contrast image. The corresponding MR image shows the beginning of cell invasion as blurriness introduced around the original artifact. By day 9, extensive cell invasion is observable both under the microscope and on the MR images.

Discussion and Conclusions

Rat C6 glioma cells can be safely and reliably labelled with superparamagnetic microbeads and single glioma cells can be detected *in vitro* in 3DFIESTA images acquired with our optimized micro-imaging system at 1.5 T. When labelled multi-cellular tumour spheroids are implanted in collagen type I gel, cell invasion can be observed with both MRI and optical microscopy. Typical cell tracking studies have followed groups of cells. This is the first work that has alluded to single cell tracking with MRI. Cellular imaging provides a new approach for the study of glioma cell invasion.

References

[1] Nygaard SJT et al. Cancer Letters 105:45-49 (1996) [2] Hinds KA et al. Blood 102(3):867-872 (2003) [3] Shapiro EM et al. PNAS 101(30):10901-10906 (2004) [4] Dodd SJ et al. Biophys J 76:103-109 (1999) [5] Hoehn M et al. PNAS 99(25):16267-16272 (2002) [6] Foster-Gareau P et al. MRM 49:968-971 (2003)