In vivo Tracking of Solitary Cells as a Tool for Comparing the Behaviour of Two Metastatically-Distinct Cell Lines in a Mouse Model of Breast Cancer Metastasis to the Brain

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Introduction: Recent studies have shown that metastatic, or secondary, brain tumours arise in 15% of metastatic breast cancer patients [1]. This has been shown to cause patients' one-year survival rate to decrease to 20% [2]. The cause of this dramatic decline in survival rate is attributed, in part, to the fact that surgery and radiation can only eradicate ~ 90% of a metastatic brain tumor [3], with 10% remaining in the brain. Remaining tumor cells are usually in the form of micro-metastases or single dormant cells which may continue to grow causing patient relapse. For this reason, work in our lab has focused on studying the dynamics of breast cancer metastasis to the brain at the cellular level. Recently we showed for the first time that individual iron-labeled brain-metastasizing breast cancer cells can be detected *in vivo* in mouse brain using a balanced steady state free precession imaging sequence and an optimized micro-imaging system on a clinical scanner [4]. In this preliminary study we investigate the potential of this cellular MR technology as a tool for understanding the underlying biological differences in different cancer cell lines by virtue of detecting and tracking individual cells from their initial arrival into the brain until their formation of metastatic tumours in the same animal. We compared a metastatic breast carcinoma cell line that was selected for its increased metastatic efficiency in the brain (MDA-MB-231BR) with its parental breast carcinoma cell line (MDA-MB-231) [5]. These cell lines will be referred to as 231BR and 231, respectively. We were able to confirm that 231BR cells were, as expected, more metastatic in the brain compared to the parental cells; unexpectedly however, our data suggest that this difference in metastatic efficiency was not attributed to differences in either initial delivery or final retention of cells in the brain.

Methods: *Cell Labeling*: 231BR and 231 were labeled with Dragon Green fluorescent MPIO beads (0.9μm, ~63% magnetite) (Bangs Laboratory, Fishers, IN). The mean iron content per cell was measured by MR susceptometry [6]. *Animal Preparation*: 6-7 week old nude female mice were anesthetized and 100,000 MPIO-labeled cells (N=3 per group) were injected into the left ventricle of the heart to deliver cells to the brain. The mice were scanned with MRI on the day of injection (day0) as well as on days 1, 3, 7, 21, and 28 post-injection. After the last time point, the mice were sacrificed and the heads fixed in formalin for 24 hrs before *ex vivo* scanning. *MRI*: Scanning was performed on a 3.0 T GE Excite whole-body MR scanner system implemented with a custom-built gradient coil insert (peak gradient strength 500mT/m, peak slew rate 3000T/m/s) and a solenoidal mouse head RF coil. 3DFIESTA images were acquired with TR/TE 14.7/7.3ms, flip angle 20°, bandwidth +/-8 kHz and 100x100x200 μm^3 resolution, which yields an average SNR in the brain of ~120 in 1 hour. To further insure the detection of all tumours on day 28, an extra set of FIESTA images with Gadolinium-enhancement was acquired with the following parameters: TR/TE 4.3/2.13ms, flip angle 20°, bandwidth +/-21 kHz, 200 μm isotropic resolution, which yields an SNR of ~100 in 7 minutes. *Data Analysis*: The number of signal voids in 30 image slices in the mid brain and the number of tumors in the brain were counted for each image data set.

Results:

Figure 1 – 231BR cells injected

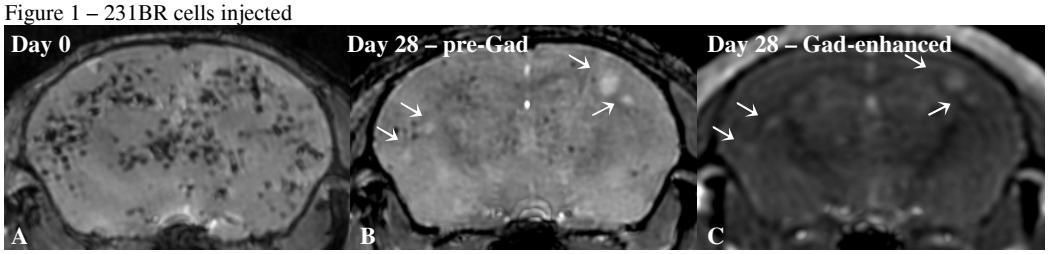


Figure 2 – 231 cells injected

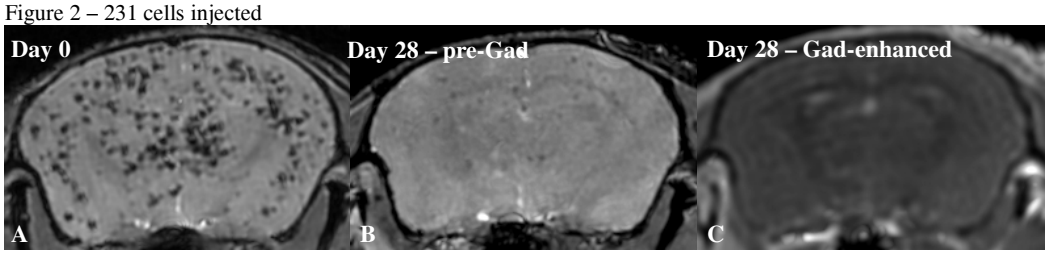
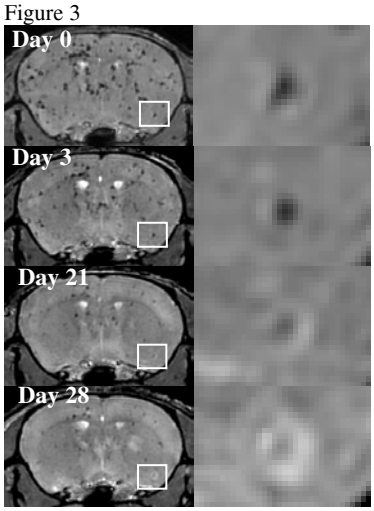


Figure 3



MPIO labeling was similar in both cell lines with an average iron content of ~57 pg/cell. At day 28, all mice injected with 231BR cells developed brain tumours (average of 26 tumours), whereas no tumours developed in mice injected with 231 cells. Figures 1 and 2 show images of mouse brains injected with 231BR and 231 cells, respectively, on days 0 (A) and 28 (B) post-injection. The presence of tumours (white arrows) was confirmed with Gadolinium-enhanced FIESTA scan (C). The average number of signal voids measured on day 0 and the number of signal voids that persisted to day 28 were not significantly different between the two cell types. Figure 3 illustrates our ability to track the growth of a tumour from a single cell, characterized by a discrete region of signal loss, over 28 days. Magnified views of areas enclosed by boxes in the left panel are shown on the right. The tumour in this example originates from a single cell that persists for the duration of the experiment. By day 28, a ring of signal hyperintensity, corresponding to tumour growth, develops around the cell.

Discussion: In this pilot study, the metastatic behaviour of different cancer cell lines were compared using a FIESTA-based cellular imaging method, which we have previously shown permits the detection of individual cells and the tracking of their metastasis formation from the single cell state. Our results suggest that the differences in metastatic efficiency observed between the two cell lines tested in this experiment is not due to either differences in the initial arrest of cells in the brain or differences in cell clearance rate from the brain. The ability to track cancer cells within an entire organ *in vivo* over time has not been previously possible with other *in vivo* imaging techniques. This powerful technology will allow us to address important biological questions about cancer cell survival and metastasis.

References: [1] Crivellari, Ann Oncol 12:353 (2001), [2] Lin, J Clin Oncol 22:3608 (2004), [3] Patchell, N Engl J Med 322:494 (1990), [4] Heyn, MRM 56:1001 (2006), [5] Yoneda, J Bone Miner Res 16:1485 (2001), [6] Bowen, MRM 48:52 (2002).

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