In vivo Tracking of Growth and Dormancy of Solitary Cells in a Mouse Model of Breast Cancer Metastasis to Brain using MRI

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Introduction

Metastasis, the spread of cancer from a primary tumour to secondary organs, is responsible for most cancer deaths. For breast cancer patients, metastasis to the brain is a significant complication that occurs in up to 30% of patients [1] and confers a one-year survival of 20% [2]. Patchell et al. reported that surgery and whole brain radiation can cure up to 90% of solitary brain metastases [3], suggesting that undiagnosed micrometastases or dormant cells are responsible for treatment failure. The ability to study micrometastatic and dormant brain metastatic tumor cells in animal models of cancer metastasis may facilitate an understanding of their biology and development of therapeutic interventions. In the current abstract we use MRI of micron-sized iron oxide (MPIO) labeled cells to track the fate of single breast cancer cells in a mouse model of metastasis to the brain. Using this technique, we were able to non-invasively visualize the initial arrest of single cells in the microcirculation of the brain and quantify the number of cells that were cleared, remained as solitary dormant cells or grew into tumours over a period of one month.

Methods

Cell Preparation: EGFP+ MDA-MB-231BR cells, a brain seeking clone of MDA-MB-231 human breast cancer cells [4], were fluorescently labeled with the cell tracking dye DiI and magnetically labeled with MPIO beads (0.9 µm, ~63% magnetite, Flash Red or Dragon Green labeled) (Bangs Laboratory, Fishers, IN). The mean cellular MPIO content was found to be ~80 pgFe/cell, assessed using a susceptometry technique [5]. In vitro growth curves were measured for unlabeled and MPIO-labeled cells. *Animal Preparation*: Cells were injected into the left ventricle of the heart of female nu/nu mice, 6-7 weeks of age (Charles River Laboratories, Wilmington, MA) to deliver cells to the brain. For metastasis endpoint studies, animals were injected with 100,000 MPIO labeled (n=8) or 100,000 unlabeled (n=8) cells. For MRI experiments, animals were injected with 10,000 (n=2), 30,000 (n=2), or 100,000 (n=2) MPIO labeled cells. *MRI*: MRI was performed on a 1.5 T GE CV/i whole-body clinical MR scanner using a custom-built gradient coil [6]. In vivo images were obtained using the 3D FIESTA pulse sequence (TR/TE 7.0/3.4ms, flip angle 30 degrees, 100×100×200 μm^3, acquisition time of 1.5 hours, SNR of 60). Animals were scanned on the day of injection (day 0) and 1, 3, 7, 14, 21, 28, and 33 days post-injection. *Microscopy*: For metastasis endpoint analysis, brains were paraffin-embedded, sectioned (4 µm) and stained with hematoxylin and eosin (H&E). To correlate single cells detected on MRI with microscopy, contiguous 25 µm frozen sections were collected and sections were imaged at low and high magnification using a Zeiss 510 laser scanning confocal microscope. *Cell fate analysis*: Correlation of MRI on day 0 with day 28 allowed the fate of cells to be assessed.

Results

Figure 1

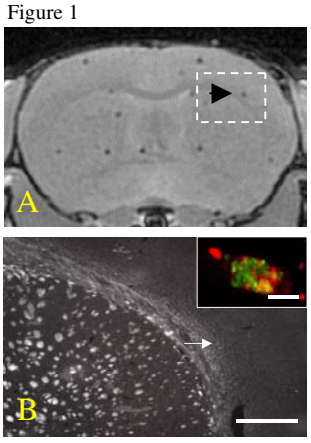


Figure 2

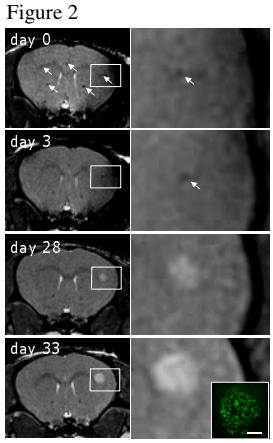


Figure 3

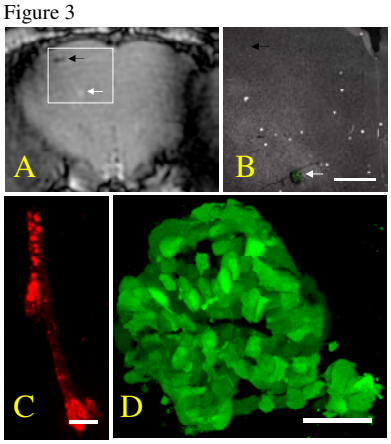
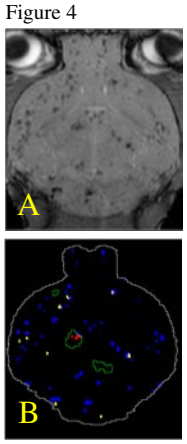


Figure 4



MPIO labeling had no effect on in vitro cell proliferation and metastatic efficiency (data not shown). MRI can detect single cancer cells in vivo (Fig. 1). MRI of mouse brain from an animal injected with 10,000 cells showed discrete signal voids on day 0 (1a), the majority of which (white box, black arrow in 1a) correlated with single DiI positive cells on optical images (white arrow in 1b, scale 500µm). Inset of 1b shows single cell with DiI (red) and MPIO/GFP (green) (scale 10µm). Longitudinal MR tracking of the growth of a single cell into a tumour (Fig. 2). MRI exhibits numerous signal voids corresponding to cells on day 0 (white arrows). Magnified view of area enclosed by box in the left panel is shown on the right. By day 3, the majority of signal voids visible on day 0 have disappeared, however, a signal void is still detectable (white arrow). On day 28 and day 33, an area of signal hyperintensity corresponding to a GFP+ tumor (inset, scale 400µm) was detected in the area previously occupied by a signal void on day 0 and day 3. MR detection of non-proliferating dormant cells and tumours (Fig. 3). In vivo MRI of mouse brain 28 days post injection show the presence of persistent signal voids (black arrow in 3a) as well as signal hyperintensity (white arrow in 3a) which correlate with a solitary dormant DiI positive cell and GFP+ tumour respectively in the low magnification (3b, scale 750µm) and high magnification (3c, scale 5µm, 3d, scale 50 µm) confocal images. MR tracking of tumour cell fate (Fig. 4). Axial MRI from a mouse injected with 100,000 cells on day 0 (4a) and corresponding cell fate map for these cells (4b). The brain is outlined in white. Blue areas correspond to "transient tumour cells" that are visible on day 0 but disappear by day 28. Green outlines correspond to boundaries of tumours visible on day 28. Red areas correspond to cells that reside within the area circumscribed by the tumour boundary at day 0 and therefore the population of cells that give rise to the tumours ("proliferating tumour cells"). One tumour outlined green is associated with cells (red) within the slice shown. The other tumours are associated with cells that appear on a different MR slice (data not shown). Importantly, no tumours arose from areas with no signal voids on day 0. Yellow areas correspond to non-proliferating or "dormant tumour cells" that are visible on day 0 and day 28. The proportion of cells initially arresting in the brain that were ultimately cleared (blue), persisted as dormant cells (yellow), or grew into tumours (red) was found to be ~94%, 4.5%, and 1.5% respectively.

Conclusion: MRI allows the detection and tracking of single cancer cells non-invasively and longitudinally within a living mouse. Through correlation of cells in 3D space and over time, we were able to assess the location and quantify the proportion of cells that ultimately cleared, developed into tumours, or remained dormant. This work reports for the first time a substantial new capability of MRI for imaging multiple stages of metastasis development in a relevant rodent model, with clear application for studying the biology and drug response of brain metastases.

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] Yoneda, J Bone Miner Res 16:1485 (2001), [5] Bowen, MRM 48:52 (2002), [6] Foster-Gareau, MRM 49:968 (2003)