Monitoring the Fate of Iron-Labeled Metastatic Melanoma Cells within the Mouse Lymphatic System

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Introduction: The ability to detect cancer cells in lymph nodes using MRI would permit the monitoring of invasive cancers, studies of cancer cell tracking in the lymphatics and may lead to a better understanding of the metastatic nature of cancer. In this abstract we present data from experiments aimed at optimizing in vivo MRI technology for detecting cells, which includes a SSFP imaging protocol for high resolution imaging at 1.5T, and allows for the detection of as few as 100 iron labeled melanoma cells within the mouse inguinal lymph node.

Methods: *Cell labeling.* B16F10 melanoma cells were labeled with magnetic nanoparticles (MNP) (Bangs beads, 0.9 μm). Iron content was measured by susceptometry and ICP-MS. The lifespan of the MNP in cells was evaluated by staining with Perl's Prussian blue and by measuring the iron content in cells at various time points after labeling (1,3,5,10,13 days > labeling). *Lymph node imaging.* A 5 μl suspension of labeled B16F10 cells was injected into the right inguinal lymph node of C57Bl6 mice using a Hamilton syringe. Two experiments were performed: a longitudinal study and a cross-sectional study. In the longitudinal study, mice were injected with different numbers of labeled cells (100 cells; n=6, 1000 cells; n=10, 10,000 cells; n=20) and then imaged repeatedly on days 1, 4, 7, 14 and 21 post injection. Control groups received saline (n=2), free MPIO beads (100,000, n=2) or unlabeled cells (10,000, n=3). In the cross-sectional study, 5 groups of 3 mice (total n=15) were injected with 10,000 labeled cells and the separate groups of mice were imaged once at 5 different time points (days 1, 8, 10, 12 and 15) and then sacrificed for histological analysis. MRI was performed on a 1.5 T GE EXCITE whole-body clinical MR scanner using a custom-built gradient coil and mouse body solenoid RF coil. *In vivo* images were obtained using the 3D FIESTA pulse sequence. The optimized sequence employed TR/TE = 9.1/4.5 ms, flip angle = 30 degrees, bandwidth = 21 kHz and phase cycling. Images were acquired at either 200 μm^3 or at 100 μm^3 isotropic spatial resolution. Acquisition times were either 34 or 108 minutes.

Results: *Cell labeling.* B16F10 cells were readily labeled with MNP while maintaining high viability. The cellular iron content of the injected cells was ~35 pgFe/cell. Serial photomicrographs of Perl's stained cells showed the disappearance of iron within dividing cells; no iron was detected in cells by Perl's staining at 10 days after labelling (~8-10 divisions). The results of iron quantification support the histological results; at 10 days after labelling iron content was < 1 pgFe/cell.
Lymph node imaging. **Figure 1** shows the effect of employing RF phase cycling. In 1A phase cycling is not used. Significant banding artifacts are produced in FIESTA images of the mouse body where many different tissue interfaces exist (arrows). Banding at the body surface is especially detrimental to imaging of the lymphatic system since the inguinal node is adherent to the skin's surface. Figure 1B shows that the ability to visualize the inguinal node is improved dramatically when phase cycling is implemented. Whole mouse body images were acquired in ~30 minutes. Ten pairs of lymph nodes could be easily identified in various slices from the 3D data sets; including the popliteal, iliac, inguinal, renal, axial and brachial nodes. This is facilitated by the very high contrast between the normal nodes and the surrounding fat (CNR ~65). The volumes of the normal inguinal and popliteal nodes were measured to be 3.20 (± 0.81) mm^3 and 0.79 (± 0.36) mm^3, respectively.

Regions of signal loss were observed within the nodes of mice injected with labeled melanoma cells. In **Figure 2** MR images of the nodes, on day 1 post injection, are shown cropped and enlarged. A= saline injection, B= 100 cells, C= 1000 cells, D=10,000 cells. These images were acquired at a resolution of 200 μm^3. Blinded observers could not tell the difference between images of nodes injected with saline (2A) or with 100 labeled cells (2B). Focal regions of signal loss were observed within the nodes in mice injected with 1000 or 10,000 cells (2C&D). In a subsequent group of mice injected with 100 cells (n=5), images were acquired in the axial plane with a higher spatial resolution and longer scan time. **Figure 3** shows that in these images, with 100 μm^3 resolution, small focal patterns of signal loss could be observed (arrow). The same observation was made in 4/5 mice injected with 100 labeled melanoma cells and the signal loss persisted for 22 days post injection. Tumor growth could be detected in the MR images (**Figure 4**), by day 14 post-injection in 5/10 mice injected with 1000 cells. In mice injected with 10,000 cells, tumors were visualized in images by day 7 in 2/5 and by day 14 in 5/5 mice. Tumors did not develop in mice injected with 100 cells. It is interesting to note that in all mice, which developed nodal tumors after injection of labeled melanoma cells, regions of signal loss persisted during the 4 weeks of imaging. In mice injected with 10,000 unlabeled melanoma cells tumors developed at the same rate but signal loss was not observed in the tumors.

Discussion: Our cellular MRI techniques allow for the visualization of mouse inguinal lymph nodes, and most importantly, the detection of as few as 100 iron-labeled melanoma cells transplanted in to the inguinal lymph nodes of mice. This technology allows us to track tumor cell growth and offers the possibility of being able to detect small numbers of cells that have metastasized to distant lymph nodes. Whole mouse body images can be acquired, in reasonable scan times, which allow the visualization of 10 pairs of nodes simultaneously. *In vitro* results suggest that the MNP label would be diluted beyond detection by cell division after ~5 days for melanoma cells. However, our image data shows that signal loss persists within growing tumors, suggesting that the dynamics of cancer cell division and label dilution is much more complex *in vivo*.

Figure 1.

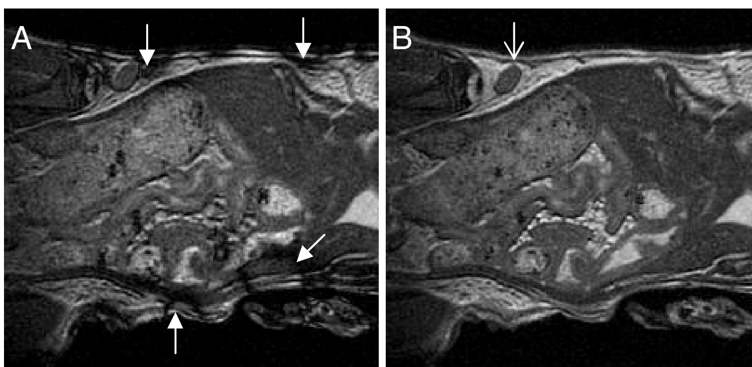

Figure 2.

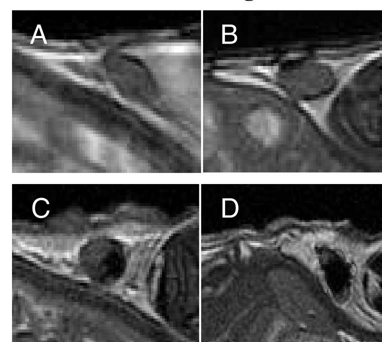

Figure 3.

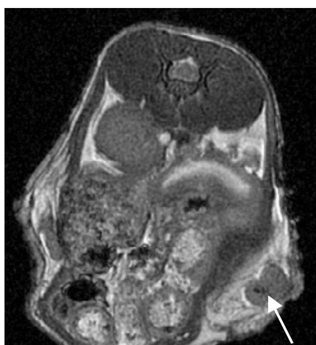

Figure 4.