Imaging the Metastasis of Cancer Cells From a Primary Implant Site

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INTRODUCTION: The role of the lymphatics as a mode of tumor cell transport has been known for some time and remains of critical importance since most cancer deaths result from metastatic dissemination away from the primary tumor. The detection of cancer cells was one of the early applications of cellular MRI. There are many examples of the use of MRI and iron-based cell labels for imaging of primary, implanted tumors. There are also examples of the detection of metastatic cancer after the systemic (intravenous or intra-arterial) administration of iron-labeled cells. To the best of our knowledge there are no prior reports of the use of cellular MRI to identify iron-labeled cancer cells that have metastasized from a primary, implanted tumors – true metastasis.

In the experiments described here, we used a unique mouse model of metastasis, where iron-labeled melanoma cells are implanted directly into the inguinal lymph node, and whole mouse body imaging. The cells (B16F10) are known to be highly metastatic and to metastasize via the lymphatic system. In this paper we show, for the first time, the ability to detect cancer cells that have metastasized to draining nodes via the lymphatic system, after tumor development at the implantation site.

METHODS: ***Cell labeling:*** B16F10 cells were labeled with micron-size iron-oxide particles (MPIO) (diameter~0.9 µm, Bangs Labs). Iron content was measured using a colourimetric assay. ***Animal Model:*** 10^4 iron-labeled cells were implanted directly into the right inguinal lymph node of C57Bl/6 mice (N=12) using a specially designed 30G microneedle (Becton Dickinson Technologies, NC). ***MRI:*** All scans were performed on a 1.5T GE clinical MR scanner using a custom-built gradient coil (maximum gradient strength=600 mT/m, and peak slew rate= 2000 mT/s). A whole mouse body solenoid RF coil was built to allow simultaneous imaging of all nodes for tracking metastasis. Images were obtained using the 3D fast imaging employing steady state acquisition (3D-FIESTA) pulse sequence (TR/TE= 9.22/4.16 ms, flip angle= 30°, NEX=2, resolution=200 μm^3 isotropic, scan time=23 min). Phase cycling was employed to minimize banding artifact in the mouse body images. Scans were taken on days 1, 7, 10, 14, 21 and 28. ***Histology:*** The implanted node/tumor and the axillary nodes of interest were removed and fixed. Contiguous 10 µm tissue sections were stained with H&E for node structure, Pearl's Prussian Blue for iron and S100 for melanoma cells.

RESULTS: Mean cellular iron content was ~ 30 pgFe/cell. Direct implantation of cells into the lymph node via microneedle did not affect cell viability or lymph flow. 3D-FIESTA images of the mouse body allowed the visualization of multiple lymph nodes with high fat-node CNR. In 10/12 mice signal loss was observed within the implanted inguinal lymph node on day 1 post-implantation (Figure 1). In 9 of these 10 mice, signal loss persisted in the implanted node as the tumor developed, becoming dispersed throughout the tumor tissue. In 5 of these 10 mice, signal loss was also observed in the first draining lymph node, the axillary node (Figure 2). The development of metastatic tumors in the axillary nodes could be observed by MRI in 4/5 mice. Iron-labeled melanoma cells were identified by histology in the axillary node and in developing inguinal node tumors.

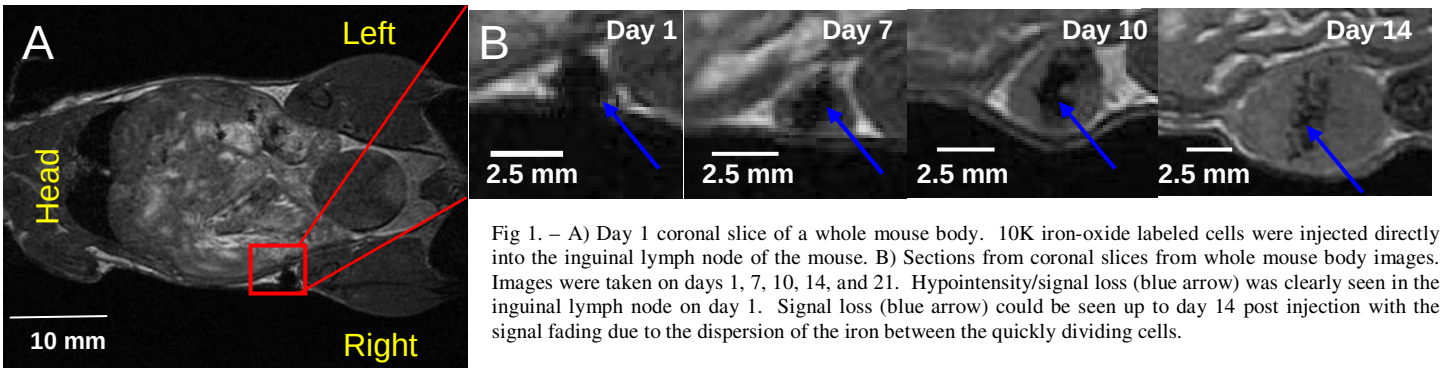

Fig 1. – A) Day 1 coronal slice of a whole mouse body. 10K iron-oxide labeled cells were injected directly into the inguinal lymph node of the mouse. B) Sections from coronal slices from whole mouse body images. Images were taken on days 1, 7, 10, 14, and 21. Hypointensity/signal loss (blue arrow) was clearly seen in the inguinal lymph node on day 1. Signal loss (blue arrow) could be seen up to day 14 post injection with the signal fading due to the dispersion of the iron between the quickly dividing cells.

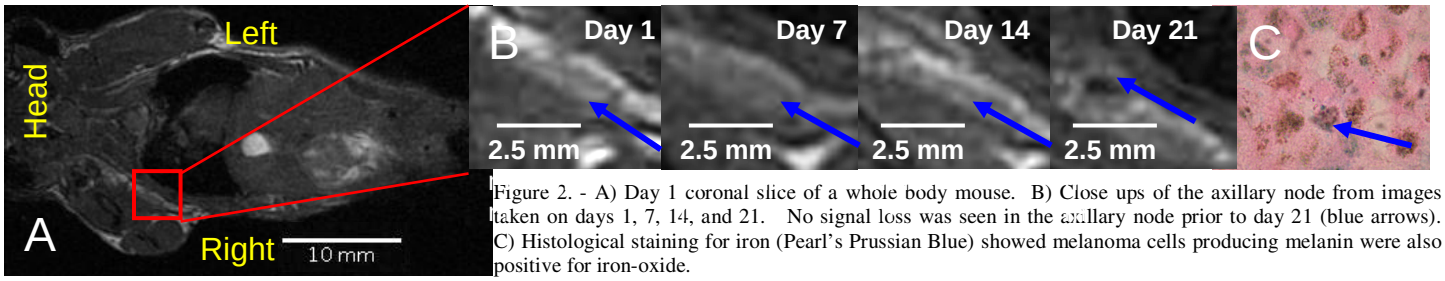

Figure 2. - A) Day 1 coronal slice of a whole body mouse. B) Close ups of the axillary node from images taken on days 1, 7, 14, and 21. No signal loss was seen in the axillary node prior to day 21 (blue arrows). C) Histological staining for iron (Pearl's Prussian Blue) showed melanoma cells producing melanin were also positive for iron-oxide.

DISCUSSION: Our results show that cellular MRI can be used to detect metastasis of cancer cells within the lymphatic system in mice. Signal void was observed in the draining axillary node after implantation of iron-labeled cancer cells in the inguinal node. Histology confirmed the presence of iron positive melanoma cells in the draining node. Several factors play into our success. Benefits of our direct intranodal mouse model of metastasis include: a reproducible method for loading of a known, low cell number into the lymph node, predictable timing of tumor development in the implanted node and direct draining to a single site, the axillary node. Our unique micro-imaging system combines the use of a 1.5T scanner and a high performance gradient insert for fast, high resolution imaging with 3DFIESTA. This combination allows us to produce high quality whole mouse body images in less than 30 minutes for visualization of all nodes and permits the detection of small numbers of iron labeled cells within the nodes. This technology allowed us to previously demonstrate in vivo detection of single iron-labeled cells at 1.5T.

Iron-based labels are generally considered limited for imaging proliferating cells because of the predicted loss of label with cell division. However, cancer cells are not normal and cancer cell division in vivo is not predictable. In our lab we have observed retention of the iron label in cells in various cancer models. Unequal cell division and unequal distribution of iron particles between dividing cells is likely the reason for retention of label (unpublished results). Still, a permanent predictable MR cell label would be better. The use of endogenous iron reporters and/or targeted agents should further improve our cancer imaging capabilities.