MRI detection of brain metastases labeled with iron oxide nanoflowers

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Target audience – Breast cancer and metastatic cancer researchers

Purpose – Breast cancer is among the types of cancer with a high propensity to metastasize to the brain. While improvement in treatment of primary tumors has overall increased the survival of breast cancer patients, patients diagnosed with brain metastases typically survive only 2-16 months after the diagnosis. Understanding mechanisms of breast cancer metastasis to the brain is therefore an important research area for improving patient outcomes.

Methods – Fe_3O_4 nanoflowers were synthesized as published previously.[1] Human breast carcinoma cells (MDA-MB-231) were transfected with mCherry fluorescent and luciferase reporter genes using a retroviral vector. Cells were incubated with 50 mg/mL of Fe_3O_4 nanoflowers for 24h; then $2x10^5$ cells/mouse in 200 µl of PBS were injected into the left cardiac ventricle of 4-week-old female athymic nude mice. Mice were screened for successful intracardiac injection using bioluminescence imaging on an IVIS Spectrum. Within 45 min. of injection, mice received 150 mg/kg D-luciferin Firefly intraperitoneally and bioluminescence images were acquired 12 min. later. Only mice with bioluminescence detected in brain and bones, but not lungs, proceeded to MRI.

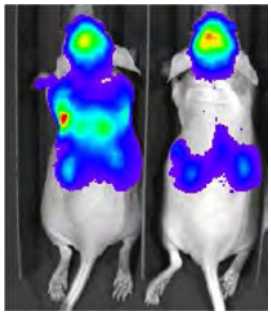

Figure 1: Representative images of mice excluded (L) and included (R) in the MRI study based on IVIS screening.

MRI was performed on a 7T Bruker PharmaScan 7 days post tumor cell injection, after confirming the presence of tumor cells in the brain using bioluminescence as described above. Mice were anesthetized with isoflurane and positioned in a 23mm quadrature volume coil designed for mouse brain. Respiratory gated 3D gradient echo (FLASH) images were acquired with TR/TE/α = 20 ms/6 ms/7°, field of view = 20x15x17.5 mm^3, and matrix = 167x125x146 for an isotropic resolution of 0.120 mm. Scan time was ~13 minutes depending on gating efficiency and 3 repetitions were acquired. The scan repetitions were averaged, then the brain was segmented in Amira 5.4. Iron labeled cells appearing as signal voids on the image were manually segmented on each slice by an experienced observer, taking care to avoid including large blood vessels with similar appearance. A connected component analysis was used to avoid overcounting metastases appearing on more than one image slice.

After imaging, animals were transcardially perfused, and brains were embedded in Optical Cutting Temperature medium, frozen, and sliced 10 µm thick for Prussian Blue iron staining.

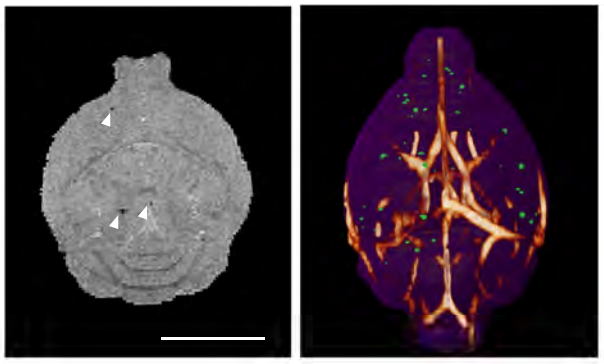

Figure 2: (L) Single slice of skull-stripped MRI showing metastases, scale bar 5mm; (R) 3D rendering of brain surface and metastases (green) overlaid on angiogram.

Results – The Fe_3O_4 nanoflowers had an average size of 162nm as measured by dynamic light scattering, and an average r_2 of $207s^{-1}mM^{-1}$ at 7T. Six mice with successful intracardiac injections as determined by bioluminescence imaging were included in the MRI study (**Figure 1**). Labeled cells were visualized as signal voids on the MR images and were distributed throughout the brain (**Figure 2**). An average of 37±25 metastases were detected across the study group. Prussian Blue staining shows individual nanoflower-labeled cells located near blood vessels (**Figure 3**).

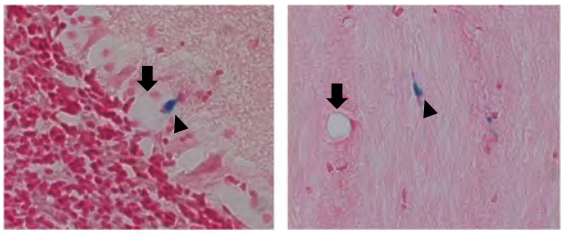

Figure 3: Prussian Blue iron staining suggests detection of single cells (arrowheads) in the vicinity of blood vessels (arrows). 40x magnification.

Discussion – Iron nanoflower-labeled brain metastases were readily detected in all animals. Cell tracking with these highly sensitive nanoflowers offers a promising approach to the study of factors controlling brain metastasis. Using bioluminescence imaging for rapid high-throughput screening of animals allows study inclusion of only animals with successful cell delivery to the brain. This platform will support a variety of future studies on the effects of cancer cell gene expression, mouse knockout models, and new therapies on metastasis growth in the brain.

References – [1] Hu F et al. *Chem Commun (Camb)*. 2010 Jan 7;46(1):73-5.