

Quantitative Measurement of Cerebral Metastatic Tumor Burden of Breast Cancer Cells in a Nude Rat Model Using T2* Map Histograms with Bioluminescence Imaging Correlation.

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Introduction Detection of single cells or small numbers of tumor cells would be a valuable tool to investigate the course of metastatic disease. *In vivo* tracking of tumor cells by MRI is technically feasible by labeling cells with superparamagnetic iron oxide particles (SPIO). However, even with SPIO labeled cells, MRI quantitation of tumor burden is complicated by low signal to noise ratios, partial volume effects, and spatial resolution. Localizing the tumor cells in the region or organ of interest and measuring total metastatic tumor burden in a quantitative manner has not been done.

Purpose The purpose of this study was to investigate whether cerebral metastases of a SPIO-labeled breast cancer cell line containing the luciferase gene could be quantitated using T2* histogram maps obtained by MRI imaging at 3T. The T2* histogram data was correlated with bioluminescence imaging and with histopathology of the brain lesions.

Methods A Feridex-Protamine sulfate (FePro) labeled MDA-MB-231BR-Luc human breast cancer cell line was injected into 6- to 7-week-old nude rats. Rats underwent intracardiac infusion of 1 to 3 x 10⁶ cells and imaging was performed at days 1 - 3, and weeks 1 - 4. MR scanning was performed on a 3-Tesla Intera (Philips Medical System) MRI in a solenoid 4 cm rf coil (Philips Research Laboratories, Germany). Pulse sequences used were: T2w TSE coronal, TR/TE 3200/60 ms, slice thickness 0.5 mm, FOV 50 mm, matrix size 224 x 512, NAV 8, reconstructed resolution 100 x 100 um; T2* map using multi-shot EPI multi slice coronal, TR/TE shortest, flip angle 30°, slice thickness 0.5 mm, matrix size 176 x 256, NAV 2, reconstructed resolution 200 x 200 um. Histograms were post-processed from T2* map data set of each scan using MEDx image processing software and Marquardt Levenberg fitting. Control histograms from uninjected rat were obtained at each time point. Bioluminescence imaging was performed at the same time point of MRI scanning.

Results Fig A demonstrates that at day 1 after injection of labeled breast cancer cell line, the T2* histogram is shifted far to the left of the normal control histogram obtained from the uninjected animal (25.3%). The abnormal T2* histogram gradually reverts back to the normal control histogram profile by week 1 (1.9%) and shifts to the right of the normal histogram by week 2. Bioluminescence signal intensity from the metastatic brain lesions also decreases by week 1 and reverts to higher values after week 2 (Fig B). Prussian blue staining revealed no detectable iron in the tumor cells or mass lesions after week 1. After week 2, multiple mass lesions (200 um in size) developed and these lesions were detectable by standard MR T2w imaging.

Discussion MR T2* map histogram, bioluminescence imaging (BLI) and histopathology of the cerebral metastatic lesions were closely correlated. Only a small portion of metastasized tumor cells survive and evolved into a tumor mass lesion. MR T2* map histogram analysis successfully showed this early engraftment and long term tracking of tumor development. This quantitative analytic tool might be useful and clinically feasible for the *in vivo* tracking of stem cell therapy and gene therapy for human trials.

Conclusion The natural history of metastasis and development of metastatic brain tumors by MDA-MB-231BR-Luc human breast cancer cells in the nude rat was monitored by T2* MRI. Quantitation by T2* map histograms correlated with BLI and histopathology. FePro labeling of cells and MR T2* map histogram should provide robust analytic tool for cellular tracking studies.

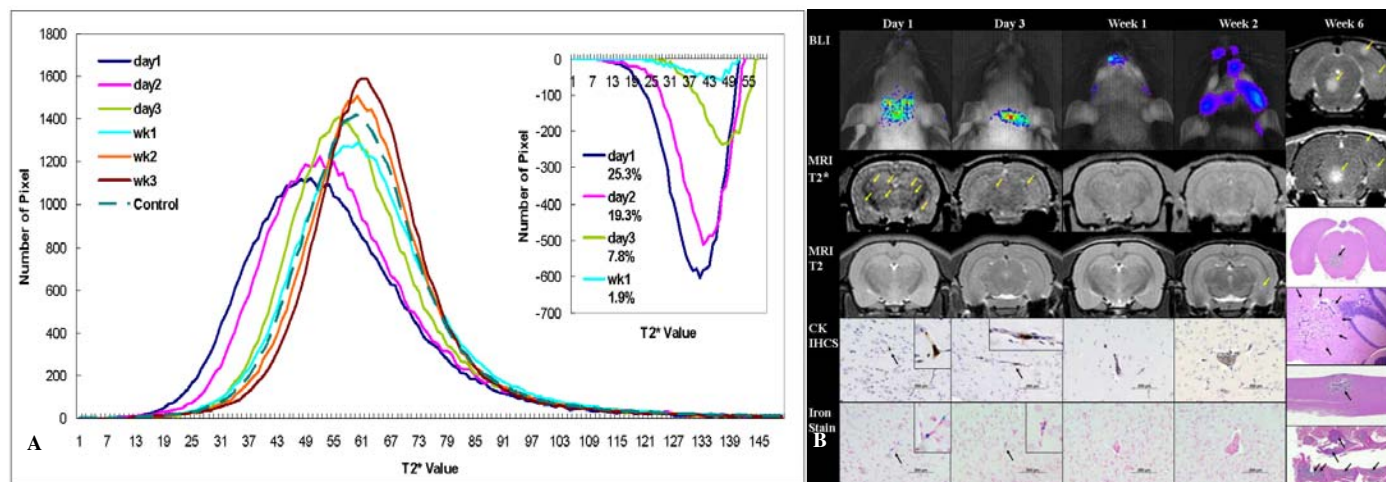


Figure Legends

A. The area under each histogram curve represents the normalized number of pixels measured by an ROI drawn on the T2* map of whole brain. The composite histogram shows the histogram shift resulting from FePro labeled metastatic tumor cells. At week 1, histogram closely approached the normal control graph (dotted line). At week 2, histogram shows a shift to the right of the normal control. This finding is closely related to the MR imaging finding and histopathological finding according to the tumor development. **Inset** shows histograms of injected animals subtracted from normal control. The percentage shift relative to the control histogram is presented

B. BLI demonstrated high photon flux from labeled MDA-MB-231BR-luc in the brain region at day 1 and day 2. Tumor activity was decreased below the threshold level of detection on the brain region at week 1. BLI photon flux activity returned in the brain by week 2.

MRI. MR T2* weighted image of day 1 shows numerous hypointense regions corresponding to FePro labeled MDA-MB-231BR-luc cells in the brain (arrows). Hypointensities were no longer appreciated by 1 week following injection of labeled cells. Turbo spin echo T2 weighted images shows increased signal intensity lesion at the left hippocampus at week 2 (arrow).

Histology. Cytokeratin immunohistochemical staining of the brain show brown colored tumor cells attached and lodged in the micro vasculature of the brain at the early period of metastatic event (day 1 - 3). Tumor grows to over 200 um in size at week 2. Prussian blue iron staining show compatible finding with CK immunohistochemical staining. Iron staining of the tumor lesions were negative after week 1.

Week 6. T2w1 and gadolinium enhanced T1w1 show multiple metastatic tumor mass lesions in the brain. Histopathology confirmed tumor mass lesion in the brain cortex, the medulla, cerebellum, spinal cord, and spinal vertebral bodies.