

Non-invasive visualization of differential BBB permeability and in vivo quantification of tumor volume in an experimental model of breast cancer metastasis to the brain, using Gadolinium-enhanced MRI and 3D bSSFP

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Introduction: Brain metastases are evident in up to 10-20% of all metastatic breast cancer patients (1). Despite a high clinical prevalence, brain metastases remain very difficult to treat, with mean survival on the order of 3-6 months, even with an aggressive treatment regime. This is in part due to the blood-brain-barrier (BBB), which can inhibit delivery of blood-borne chemotherapeutics, as well as prevent diffusion of contrast agents into a brain tumor, thus complicating detection and treatment. While others have shown that considerable heterogeneity exists in the permeability of the local BBB among brain metastases using *in situ* histology and autoradiography (2), we are the first to investigate this process non-invasively, *in vivo* over time. This was accomplished using gadolinium (Gd) enhanced MRI, to evaluate BBB breakdown, together with 3D high resolution whole brain anatomical MRI to measure brain tumor burden in a mouse model of breast cancer metastasis to the brain.

Methods: In vivo BBB permeability using MRI: A 0.1mL suspension of 1.75×10^5 MDA-MB-231-BR green fluorescent protein (GFP) and iron (BangsTM) labeled human breast cancer cells were injected into the left ventricle of the beating heart in female nude mice. Mice were imaged using a 3T GE clinical scanner with a high performance insert gradient and a solenoid radio-frequency mouse head coil. Mice were imaged with T1w SE (resolution: $117 \times 117 \times 400 \mu\text{m}$, TR/TE = 600/20, flip angle = 35°) 60 minutes post intraperitoneal injection of MagnevistTM (Gd-DTPA) on days 19, 24 and 29 post cell injection, in order to visualize BBB permeability. bSSFP (resolution: $100 \times 100 \times 200 \mu\text{m}$, TR/TE = 8/3, flip angle = 35°) was used on the same mice on the next day (days 20, 25 and 30) in order to visualize the total number of brain metastases. Tumor volumes were measured from bSSFP images. **Ex vivo BBB permeability using fluorescence microscopy:** Mice were injected with 1.5mg of 3kD Texas Red dextran in saline iv and perfused 20 minutes later. Brains were cut in 20um sections and BBB permeability was visualized by dextran leakage (red fluorescence) and tumor cells were visualized by GFP.

Results: Brain metastases are clearly visible in bSSFP images, which have contrast related to T2/T1 (Fig 1). Metastases were detected in bSSFP images at all timepoints, however, Gd enhancing metastases were only detected at mid and late time points in SE images (Fig 2A,B). The number of enhancing metastases increased with time, however at each timepoint the number of metastases detected by bSSFP was significantly greater than the number of enhancing metastases. The average volume of enhancing metastases was significantly larger than that of non-enhancing metastases (2C); although large, non-enhancing metastases and very small enhancing metastases were both observed. Enhancing metastases corresponded to brain regions with significant dextran leakage (Fig 3, red) into the brain parenchyma, in the presence of GFP positive tumor cells (green).

Conclusions: Here we have used 3D, whole brain bSSFP, which provides excellent metastasis detection without the use of contrast agent, to detect non-BBB permeable brain metastases. Furthermore, by using Gd-enhanced contrast MRI in tandem with bSSFP, we were able to longitudinally monitor the onset BBB permeability in metastases in the same animal. We are the first to observe this heterogeneous BBB permeability over time, *in vivo* in breast cancer brain metastases. Our findings indicate that the number of permeable metastases increases over time, which may help determine an optimum window for therapeutic intervention. Furthermore, by measuring metastasis volumes, we have shown that size alone is not a sufficient condition for BBB permeability, which warrants further *in vivo* investigation to better understand the potential volume/permeability relationship. Our model can provide the basis for much needed preclinical studies that investigate the ability of engineered chemotherapeutics to cross the BBB, which will hopefully lead to better clinical management and treatment of brain metastases.

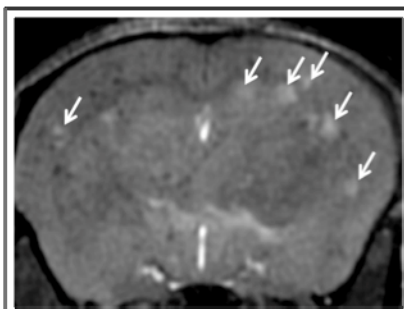


Figure 1: Axial MR image of a mouse brain using non-contrast enhanced bSSFP. bSSFP provided excellent metastasis detection (arrows) without the use of contrast agent, due to the tissue properties of the metastases and the T2/T1 contrast properties of bSSFP.

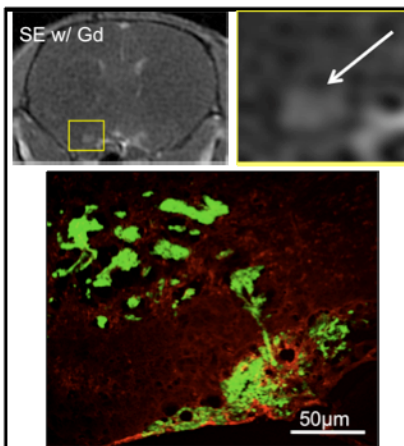


Figure 3: *Ex vivo* dextran (red fluorescence) indicates extensive BBB permeability around GFP+ (green) tumor cells in the brain. This agrees with Gd-enhanced T1w SE MRI.

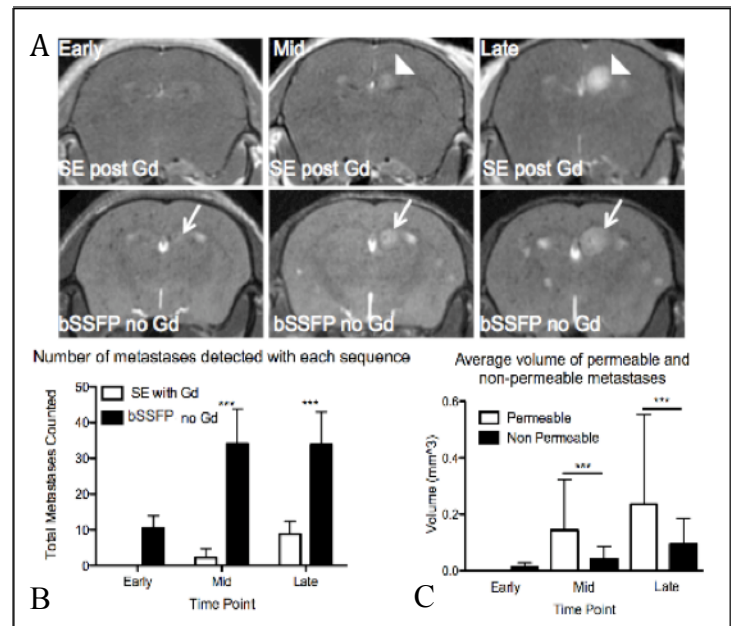


Figure 2: (A) axial images of the same animal at early, mid and late time points with both Gd-enhanced T1wSE (top) and non-contrast bSSFP (bottom). Arrow highlights a large metastasis that became permeable at mid time point (arrowhead) and remained permeable. (B) the number of metastases detected with bSSFP was significantly higher than detected by T1wSE at each time point. No Gd-enhancing metastases were detected at early time point. (C) the average volume of permeable metastases was significantly larger at mid and late time points.

References:

1. Palmieri D, *et al.* Brain metastasis of breast cancer. *Breast Disease*. 2007;26:139-47
2. Lockman PR, *et al.* Heterogeneous blood-tumor barrier permeability determines drug efficacy in mouse brain metastases of breast cancer. *Clin Cancer Res*. 2010 [Epub ahead of print]