Hypoxia Detected with Phase Contrast MRI is an Early Event in Micrometastatic Breast Cancer Development in the Rat Brain

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Introduction
Hypoxia is a driving force in the angiogenic cascade that ultimately leads to increased tumor growth. Blood oxygen level dependant (BOLD) MRI, or T2*-weighted contrast, is related to the oxygenation status of tumors. However, R2* (1/T2*) measurements in brain tumors can be affected by changes in R2 related to edema in addition to those caused by magnetic field inhomogeneities (R2') from deoxygenated hemoglobin. On the other hand, phase contrast MRI is exquisitely sensitive to the magnetic susceptibility properties of brain tissues. The purpose of this study was to determine if phase contrast MRI was more sensitive to vascular abnormalities than BOLD contrast in brain metastases in a rat model of breast cancer metastases to the brain and whether these changes were indicative of hypoxic changes that precede angiogenesis.

Methods
10⁶ MDA-MB-231-BR-eGFP³ cells were labeled with ferumoxides and protamine sulfate (FePro) and delivered to 8 week old NIH-rnu rats by intracardiac injection. Rats underwent weekly brain MRI in a 7T Bruker Biospec under a carbogen (95/5 % O2/CO2) isoflurane gas mixture. A multiple gradient-echo (MGE) sequence (TR=2500 ms, TE=3.5 ms first-echo and 4.25 ms echo spacing, 12 echoes), a T2-weighted spin-echo sequence (TR=5000 ms, TE=50 ms), and a T1-weighted gradient echo sequence (TR=400 ms, TE=3.5 ms, performed before and after intravenous delivery of 0.3 mmol/kg Magnevist) were acquired with identical spatial resolution (20 slices, 0.5 mm slice thickness, 117 μm2 in-plane). Magnitude images for all scans were computed as the absolute value of the complex data, and phase images were computed for the MGE sequence using homodyne filtering of the complex k-space data. Animals were euthanized at 5 weeks post-injection, and fixed sections underwent immunostaining.

Results
Brain metastases were clearly visible as T2 hyperintensities. These tumors were highly conspicuous on the corresponding phase images, but displayed different spatial patterns (Fig 1). Metastases were less apparent or indistinguishable on T2*-weighted images, likely due to the competing effects of T2 and T2'. Only a subset of the tumors displayed leakage of gadolinium contrast agents. Regions of interest encompassing tumors at week 5 were applied to the registered images from all previous weeks. The standard deviation of the phase within these ROIs was a reliable marker of developing metastases, with changes occurring as early as week 3 and reaching significance (p<0.05) at weeks 4 and 5 (Fig 2). The mean phase within the ROIs did not change (data not shown). T2*-weighted signal intensity and gadolinium enhancement were significantly greater in metastases at the latest time points (week 5), whereas T2* was not significant at any time point. Metastatic brain tumors grew through vessel co-option, as clusters of tumor cells along the perivascular space (Fig. 3) accompanied by edema and HIF1α (hypoxia) positive staining.

Discussion and Conclusions
Phase contrast is a sensitive indicator of brain metastases that is likely related to tumor oxygenation although the susceptibility differences associated with the increased water content cannot be ruled out. Phase changes in developing metastases occur early in tumor growth and precede angiogenesis. Phase contrast MRI offers several distinct advantages compared to magnitude images including reduced sensitivity to large-scale magnetic field inhomogeneities and T2 changes.

References
¹Rodrigues et al. 2004. JMRI. ²Duyn et al. 2007. PNAS. ³He & Yablonskiy, 2009. PNAS.

Figure 1. Heterogeneity among metastatic brain tumors from a single animal at 4 weeks following intracardiac injection of MDA-MB-231-BR-eGFP cells.

Figure 2. Regions of interest encompassing tumors at week 5 were mapped to images from previous weeks. The standard deviation of the phase (A) was sensitive to the presence of iron oxide labeled cells and increased in developing metastases as early as week 3, reaching significance at weeks 4 and 5. In comparison, whereas T2* contrast was not significantly different at any timepoint (B), T2-weighted signal intensity (C) and contrast enhancement (D) were only significant at week 5.

Figure 3. Metastatic brain tumor growth is through co-option of the existing vasculature. Interestingly, a significant fraction of tumor cells are HIF1α positive (brown), despite their proximity to the vasculature.