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 T_2*-weighted contrast, is related to the oxygenation status of tumors[1]. However, R_2* ($1/T_2$*) measurements in brain tumors can be affected by changes in R_2 related to edema in addition to those caused by magnetic field inhomogeneities (R_2') from deoxygenated hemoglobin. On the other hand, phase contrast MRI is exquisitely sensitive to the magnetic susceptibility properties of brain tissues[2]. 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

10^6 MDA-MB-231-BR-eGFP[3] 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 % O_2/CO_2) 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 μm^2 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 T_2 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 T_2*-weighted images, likely due to the competing effects of T_2 and T_2'. 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). T_2-weighted signal intensity and gadolinium enhancement were significantly greater in metastases at the latest time points (week 5), whereas T_2* 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.

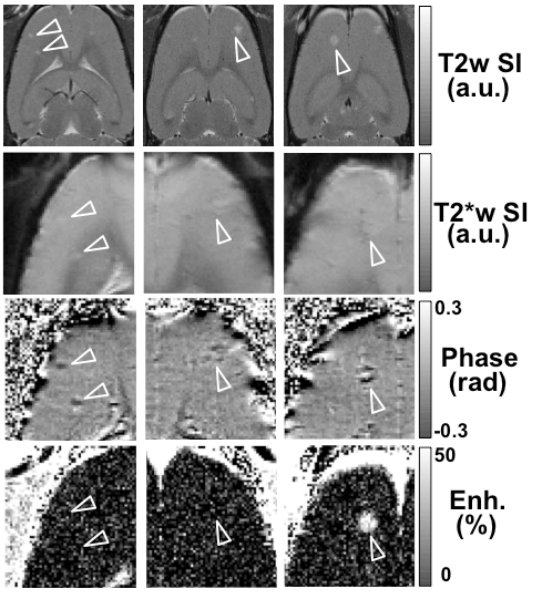

Figure 1. Heterogeneity among metastatic brain tumors from a single animal at 4 weeks following intracardiac injection of MDA-MB-231-BR-eGFP cells.

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[3]. 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 T_2 changes.

References

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

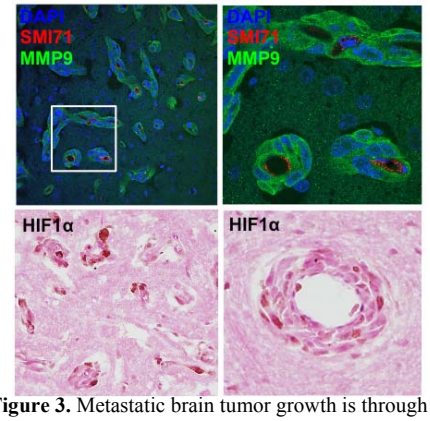

Figure 3. Metastatic brain tumor growth is through co-option of the existing vasculature. Interestingly, a significant fraction of tumor cells are HIF1α positive (brown), despite their proximity to the vasculature.

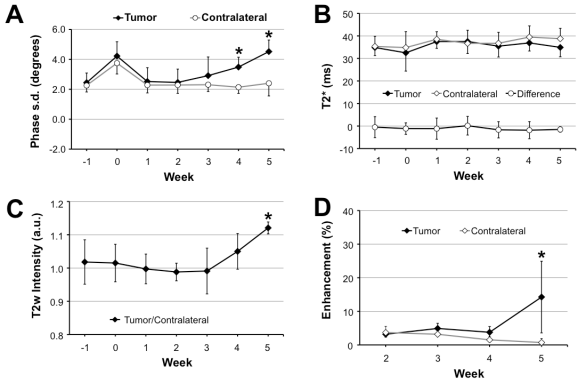

Figure 2. Regions of interest encompassing tumors at week 5 were mapped to images from previous weeks. The standard deviation of the phase (**A**) was sensitive to the presence of iron oxide labeled cells and increased in developing metastases as early as week 3, reaching significance at weeks 4 and 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.