

Molecular and Functional Imaging of Tumor Oxygenation in a Breast Cancer Model: Can Carbogen Alleviate Hypoxia?

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INTRODUCTION: Hypoxia in solid tumors arises due to the anomalous structure and function of the tumor vasculature and profoundly impacts response to therapy [1]. It also plays an important role in the manifestation of various hallmarks of cancer, including angiogenesis, altered metabolism, invasion and metastasis [2]. Blood oxygenation level dependent (BOLD) contrast MRI has often been employed to image changes in tumor oxygenation in response to administration of carbogen (95% O₂ and 5% CO₂). These studies have shown markedly heterogeneous responses to carbogen breathing, both within tumors and across tumor types [3]. While carbogen inhalation can increase oxygenation in well-vascularized tumor regions, it is not known whether it can alleviate hypoxia. To answer this question, we employed a unique combination of functional MRI and molecular imaging of a hypoxia-inducible fluorescent human breast cancer model.

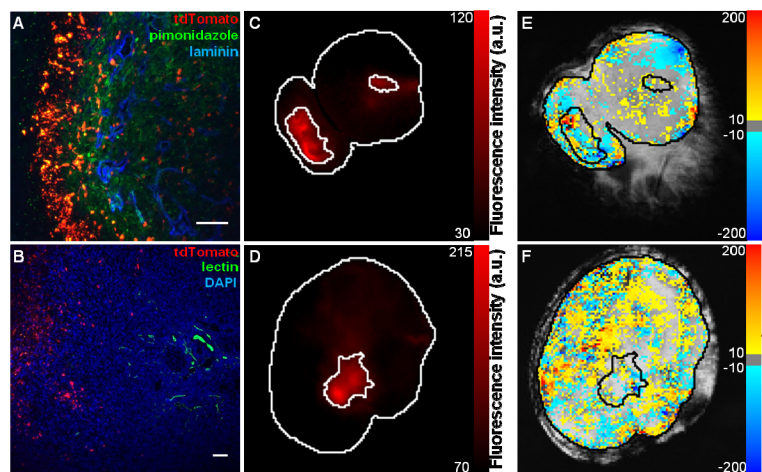


Fig. 1 Top row: tumor 1, bottom row: tumor 2. (A-B) Fluorescence images of histological sections; scale bar=100 μ m. (C-D) tdTomato fluorescence images of 1 mm-thick fresh tissue sections co-registered to ΔR_2^* maps overlaid on raw GE images (E-F). Field of view=12 mm for panels (C-F). Tumor boundaries are delineated by the outer lines and hypoxic ROIs by the inner lines.

RESULTS: Immunofluorescence showed that tdTomato expression (HIF-1 reporter) colocalized with pimonidazole adduct formation (pO₂ reporter) (Fig. 1A) but did not colocalize with lectin-FITC-labeled perfused vessels (Fig. 1B). It is noteworthy that laminin staining (basement membrane of blood vessels) showed that blood vessels were present in the pimonidazole-labeled hypoxic ROI in Fig. 1A. Fig. 1C-D shows the tdTomato fluorescence image from each tumor co-registered to the corresponding ΔR_2^* map (Fig. 1E-F). $\Delta R_2^* > 10$ s⁻¹ in only 36% of the hypoxic voxels in tumor 1 compared to 62% of hypoxic voxels in tumor 2. This is reflected in the difference between the ΔR_2^* distributions of the two hypoxic ROIs (Fig. 2), wherein the standard deviation for tumor 1 is significantly greater than that for tumor 2 ($p < 0.05$). In other words, the tumor response to carbogen, measured by the magnitude of ΔR_2^* , was greater in the hypoxic ROI in tumor 1 than that in tumor 2.

DISCUSSION: Our preliminary results demonstrate that there was high intra- and inter-tumoral heterogeneity in the ΔR_2^* responses of MDA-MB-231-tdTomato breast cancer xenografts to carbogen. This indicates that the ability of carbogen breathing to alleviate hypoxia also varies from tumor to tumor. ΔR_2^* was negative in a significant portion of the larger hypoxic ROI in tumor 1, indicating that carbogen inhalation increased oxygenation in that area. The presence of laminin stained vessels in the hypoxic area of tumor 1 (Fig. 1A) suggests that hypoxia may have resulted from poor or intermittent perfusion and not a lack of vascularity. It is possible that carbogen increased oxygenation by transiently increasing perfusion of that region. In comparison, the hypoxic ROI in tumor 2 had a significantly lower response to carbogen, indicating that carbogen did little to improve oxygenation in that region. This may be because the core of the larger tumor 2 was less vascularized (Fig. 1B). The data from tumor 2 is consistent with the hypothesis that carbogen breathing elicits a change in R_2^* in well-vascularized regions but not in poorly vascularized, hypoxic regions. However, one would expect a decrease in R_2^* due to an increase in the oxyhemoglobin concentration. In contrast, our data shows large areas of positive ΔR_2^* in both tumors. This has been observed by others and, while not fully understood, is thought result from the combined effects of blood oxygenation, blood flow and other aspects of the tumor microenvironment on R_2^* [6]. To elucidate this complex interplay, we are currently investigating the effect of carbogen on blood oxygenation and blood volume using simultaneous carbogen and iron oxide susceptibility contrast MRI, as well as novel mathematical modeling [7].

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METHODS: MDA-MB-231 human breast cancer cells engineered to express tdTomato fluorescent protein under the regulation of a hypoxia response element (HRE) promoter [4] were orthotopically inoculated into the mammary fat pad of two female athymic NCr-nu/nu mice. Tumors were imaged on a 9.4T horizontal bore scanner (Bruker BioSpin) using a T₂*-weighted multi-echo gradient echo (GE) sequence with in-plane resolution=100 μ m and slice thickness=1 mm. Images were acquired while the mice were breathing air and again while breathing carbogen. ΔR_2^* maps were computed by subtracting baseline R_2^* from carbogen R_2^* , which were computed using mono-exponential least-squares fitting. For mouse 1, 2 mg of pimonidazole (Hypoxypore) was injected i.p. before MRI for independent validation of the HRE construct. For mouse 2, 0.3 mg of BS-1 lectin-FITC (Sigma) was injected i.v. to label perfused vessels. Freshly excised tumors were cut into 1 mm sections parallel to the MR imaging plane and imaged on a fluorescence microscope to visualize hypoxic regions (tdTomato expression) before being fixed/frozen for immunostaining. Microscopy images were co-registered to corresponding MR image slices using thin-plate spline deformations [5], resampled to the MRI resolution and segmented into background, normoxic tumor, and hypoxic tumor regions of interest (ROIs) based on fluorescence intensity using k-means clustering (Fig. 1C-D). The ΔR_2^* responses to carbogen in the hypoxic ROIs were examined using histogram analysis.

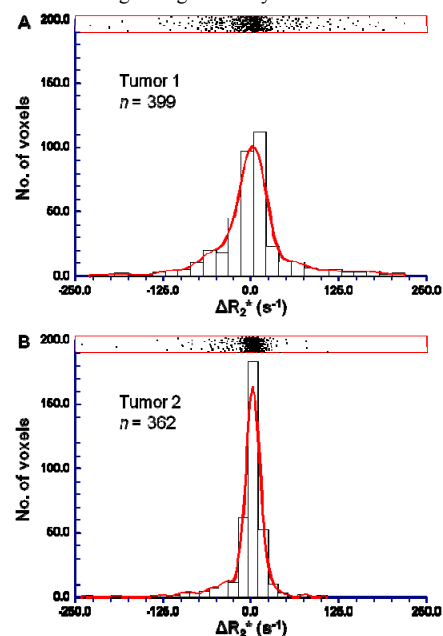


Fig. 2 Histograms and dot plots of ΔR_2^* values from the hypoxic ROIs in tumor 1 (A) and tumor 2 (B). The distribution for tumor 1 has heavier tails, whereas the distribution for tumor 2 is more closely centered around zero.