Localized Hypoxia Results in Spatially Heterogeneous Metabolic Signatures in Breast Tumor Models

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Introduction: Tumor hypoxia triggers signaling cascades that significantly impact on biological outcomes, resulting in resistance to radio- and chemotherapy. Therefore, understanding the origin and extent of hypoxia in tumors is critical. Prolonged, severe hypoxia frequently results in necrosis. In this study, we have investigated the relationship between hypoxia, necrosis, and several metabolites, such as, lactate/lipid and total choline (tCho), in a human breast cancer model by combining *in vivo* magnetic resonance imaging (MRI) and magnetic resonance spectroscopic imaging (MRSI) with *ex vivo* optical imaging of hypoxia and necrosis.

Methods: Human MDA-MB-231-HRE-tdTomato breast cancer cells, which were genetically engineered to express red fluorescent tdTomato protein under hypoxic conditions, were orthotopically grown in nude mice [1]. Both 3-dimensional (3D) water-unsuppressed MRSI to detect water and water-suppressed 3D MRSI to detect metabolites were performed. Inherently registered 3D T1-weighted images were acquired to measure the tumor anatomic structure as a reference for MRSI. Each tumor was then sectioned to obtain fiducially marked 2-mm thick slices, which were imaged by brightfield and fluorescence microscopy to visualize hypoxic tumor regions. Each 2-mm thick slice was cryo-sectioned into fiducially marked 10-um thick slices and hematoxylin/eosin (H/E) stained to visualize the necrotic area in the tumor. We performed 3D reconstruction of MRI/MRSI by using an in-house IDL program and 3D reconstruction of optical hypoxia and necrosis images of tumors based on the locations of the fiducial markers, including shape extractions and volume rendering. The 3D optical hypoxia images were registered to the H/E stained images based on the fiducial markers. The 3D optical brightfield images and the corresponding 3D hypoxic region and necrotic region were registered to high-resolution 3D tumor anatomic MRI T1-weighted images along with 3D metabolite images by combining rigid transformation and non-rigid b spline transformation (Fig A). Regions where hypoxia, normoxia or necrosis overlapped with a given metabolite, which is defined as metabolite volume percentage (Fig B), and the average metabolite concentrations (Fig C) in hypoxic, normoxic, and necrotic regions were measured.

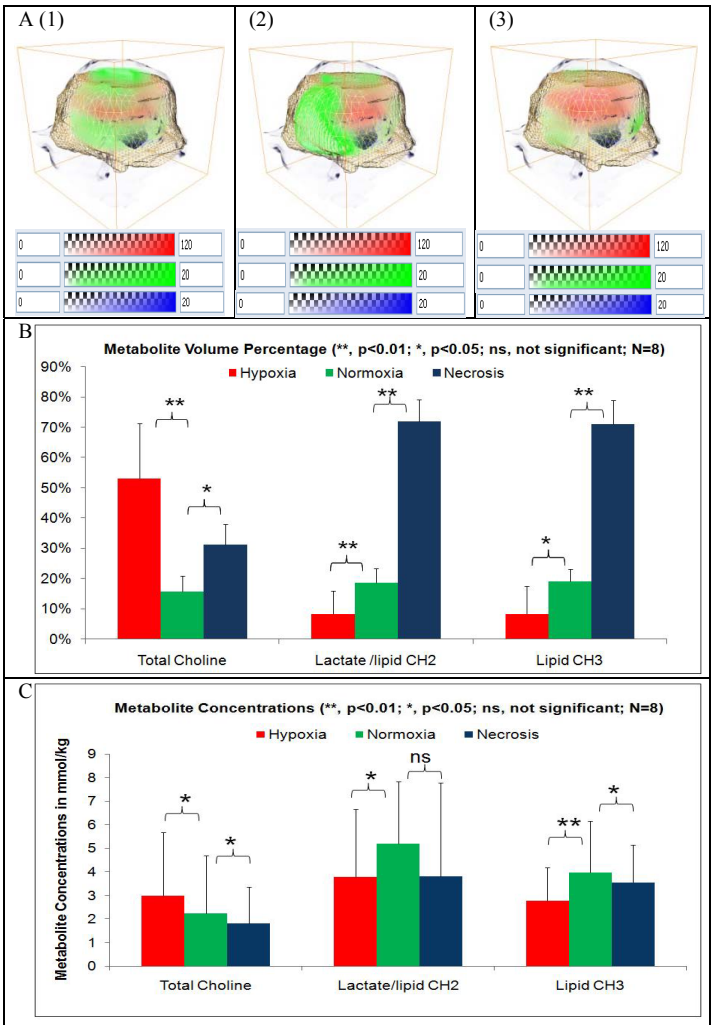

Figure (A): 3D Co-registration of MRI-detected tumor shape (light purple surface), optical images of tumor shape (grid), hypoxia fluorescence (red), necrosis (blue) and MRSI-detected metabolites (green). (1) Total Choline (green). (2) Lipid CH2 (green). (3) Lactate/lipid CH3 (green). (B) Metabolite Volume Percentages and (C) Metabolite Concentrations in hypoxic (red), normoxic (green), and necrotic (blue) tumor regions. Values are mean ± standard deviation (n=8).

Results: A co-registration and reconstruction platform based on fiducial markers and shape characteristics was built to fuse three different imaging modalities. Eight MDA-MB-231-HRE-tdTomato breast tumors, four of which contained necrotic regions, were analyzed (Fig B and C). tCho (3.2 ppm), lactate/lipid CH2 (1.3 ppm), and lipid CH3 (0.9 ppm) were detected by MRSI. Both volume percentage (Fig. B) and concentrations (Fig. C) of tCho in the hypoxic regions were found to be significantly higher than those in the normoxic and necrotic regions. Lactate/lipid CH2 and lipid CH3 volume percentages in the necrotic regions were significantly increased compared to both hypoxic and normoxic regions, while being significantly lower in the hypoxic regions than in the normoxic regions (Fig. B). The average lipid CH3 concentration in the necrotic regions was much higher than that in hypoxic regions but lower than that in normoxic regions (Fig. C). The average lipid CH3 concentration in the hypoxic regions was significantly lower than that in normoxic regions (Fig. C). The average lactate/lipid CH2 concentration in hypoxic regions was significantly lower than that in normoxic regions, however, no significant difference was detected between hypoxic and necrotic regions (Fig. C). The standard deviation of concentrations in necrotic regions was high, as the number of tumors containing necrosis was small.

Discussion and Conclusions: Necrotic regions contained predominantly voxels with lactate/lipid CH2 and lipid CH3, however the concentration per voxel was just above the threshold setting for the volume percentage calculation. However, both volume percentages and concentrations of tCho were increased in hypoxic tumor regions in our breast tumor model, which is in good agreement with data of elevated tCho under hypoxia, resulting from hypoxia-induction of choline kinase as previously observed in a prostate cancer model [2]. Increased volume percentages of lactate/lipid CH2 and lipid CH3 were previously demonstrated in necrotic tumor regions [3], which is in good agreement with our data. In conclusion, combining 3D MRSI/MRI and optical imaging proved useful to delineate the effects of tumor hypoxia and necrosis on MRS-detectable metabolites, some of which may, in the future, serve as surrogate markers for hypoxia and/or necrosis.

References: [1]. Raman, V. et al. (2006). *Cancer Res* **66**: 9929-36. [2]. Glunde, K. et al. (2008). *Cancer Res* **68**: 172-80. [3].Opstad, K. S., B. A. Bell, et al. (2008). *NMR Biomed* **21**: 677-85. Acknowledgements: We thank Tiffany B. for technical laboratory support and funding support NIH R01 CA134695.