Mapping in vivo Tumor Oxygenation within Viable Tumor using ^{19}F MRI and Multispectral Analysis

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Introduction Hypoxia in tumors represents a compelling imaging target, given that it has a major role in tumor development and resistance to therapy[1]. The ability to quantitatively measure regional tumor tissue oxygenation *in vivo* can provide valuable information about tumor physiology to aid in the development and monitoring of potential therapies. ^{19}F magnetic resonance imaging(^{19}F MRI) oximetry, using perfluorocarbon emulsions(PFCs) as an imaging contrast agent, is a noninvasive method that has shown promise for quantifying the partial pressure of oxygen(pO_2) within tumors[2,3]. However, tumor heterogeneity complicates the quantification of tissue pO_2 due to variability in the distribution of the contrast agent and the inclusion of non-viable tumor regions in whole tumor estimates. To address these issues, we developed novel approach that combines ^{19}F MRI T_1 mapping with diffusion-based multispectral K-means (KM) clustering to quantify pO_2 in specific tumor tissue populations. This study demonstrates that pO_2 measurements can be restricted to the viable tumor and that the necrotic tissue classes contribute erroneous data to whole-tumor estimates of the pO_2 response during a breathing gas challenge experiment. This approach provides a means to measure pO_2 within the viable tumor and address the issue of tumor heterogeneity that complicates pO_2 tumor imaging.

Methods MR experiments: Experiments were performed with a 9.4T Agilent MRI system equipped with a ^{1}H/^{19}F 10 mm surface coil (Agilent Technologies Inc.). 1-mm-thick coronal slices were acquired (n = 12, FOV=25.6×25.6mm, matrix=64×64). A diffusion-weighted fast spin echo multislice (FSEMS) sequence was used to calculate an apparent diffusion coefficient (ADC) map (6 b-values ranging from 270 to 1000 s/mm^2, TR=3s, ETL=4, NA=2, Δ=30ms, δ=3.3ms). A spin echo multislice (SEMS) sequence was used to generate T_2 and M_0 maps (TE = 5,26,47,68 ms, TR = 3s, NA= 1). A T_1-weighted SEMS sequence was used to obtain a fluorine anatomical reference image. A ^{19}F single-shot, inversion recovery FSEMS sequence was employed to generate spatial maps of T_1 (FSEMS, TI =0.1,0.3,0.5,0.6,0.7,0.9,1.2,1.8,2.5s, TR=6s, ESP=4.1ms, ETL=32, NA=32, matrix=32×32, zero-filled to 64 ×64). Multispectral analysis of ^{1}H data was used for tissue segmentation. K-means clustering was performed using the ADC, proton density and T_2 maps as previously described [4,5]. The K-means algorithm segmented the tumors into four tissue classes: viable tumor tissue, sub-cutaneous adipose tissue, and two necrotic classes [4,5]. The tissue class map was combined with the ^{19}F T_1 map to estimate pO_2 in the four tissue classes.

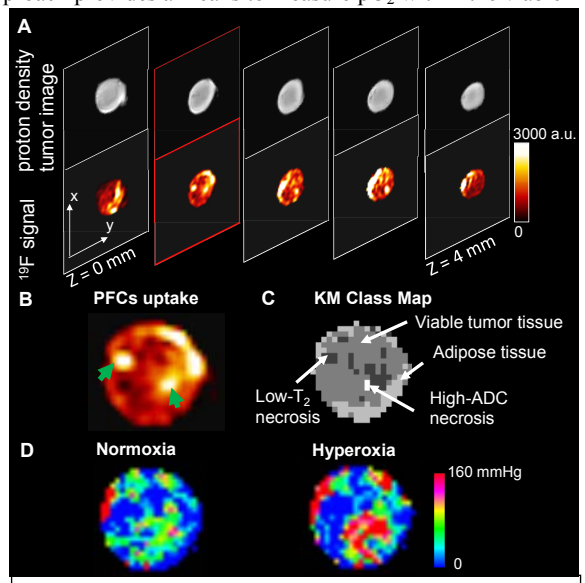

Fig.1. Representative images from one mouse. (A) multislice proton density images with co-registered PFCs intensity. (B) A sample image of PFCs uptake and (C) corresponding KM class map. (D) pO_2 maps acquired before and during a carbogen breathing challenge.

Samples and animals: The Institutional Animal Care and Use Committee (IACUC) at Genentech approved all animal protocols. Athymic nude mice (n=20) were inoculated subcutaneously on the hind limb with HM7 colorectal cancer cells. The imaging contrast agents, PFCs containing 60 w/v% perfluoro-15-crown-5-ether (Synquest Inc.) were intravenously injected into mice(400 μL/dose) at 48 h and 24 h prior to MRI, respectively. During the experiment, T_1 maps were acquired at normoxia(21% O_2) and hyperoxia(carbogen: 95% O_2, 5% CO_2) conditions sequentially by changing the breathing gas. A 10 min gap was employed between the two acquisitions to allow the oxygenation to reach equilibrium, monitored by the O2C system(LEA Medizintechnik GmbH Inc.). A calibration curve (R_1 vs O_2) was constructed by measuring the T_1 of PFCs at known concentrations of dissolved oxygen.

Results and Discussions PFC uptake was significant, but variable within the tumor following intravenous PFC injection (Fig.1A). Strong ^{19}F signal was visualized within some areas of the viable tissue class (Fig. 1BC) and is consistent with the presence of large vessels in these regions. In addition, strong ^{19}F uptake was observed in the low-T_2 necrosis class (Fig.1B, green arrows), where leakage is likely due to hemorrhage [4]. This class has been found histologically to contain intake red blood cells that shorten the T_2 and is likely an area of recent or active hemorrhage [4]. Under breathing gas challenge, there was a heterogeneous response within the tumor (Fig. 1D). Statistical analysis revealed that the viable tumor class (paired t-test, p=0.018), adipose tissue class (p<0.01) and low-T_2 necrosis class (p<0.01) exhibited a significant increase in pO_2 in response to hyperoxia challenge (Fig.2). The increase in pO_2 for the low-T_2 necrosis class provided further evidence of active hemorrhage. The high-ADC necrosis class showed no change in pO_2 (p=0.10), likely due to the acellular, "cyst-like" nature of the region [4]. The differing sensitivity in response to the gas challenge among the four tissue classes is due to the difference in pathophysiological features of the tissues. These results indicate that the inclusion of non-viable tumor tissue regions in whole-tumor estimates of pO_2 response can mask or bias the changes of pO_2 within the tissue of therapeutic interest (viable tumor). Restricting analysis of tumor oxygenation to the viable tumor is physiologically meaningful and could aid investigations of therapeutic responses.

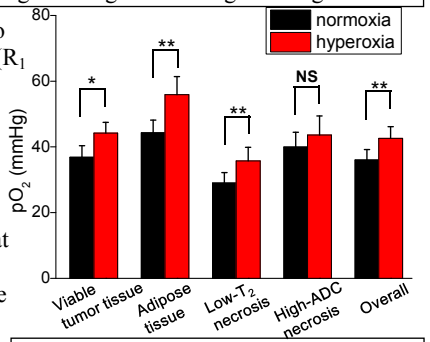

Fig.2. Mean pO_2 change from normoxia to hyperoxia (with std error).*p<0.02 **p<0.01 NS: not significant.

Conclusions This is the first study to employ a diffusion-based multispectral tissue segmentation approach to address the complications of tissue heterogeneity in ^{19}F MRI pO_2 mapping. This study has demonstrated that this approach can detect tissue dependent oxygenation changes in response to a breathing gas challenge.

References [1] William and Michael, Nature Reviews Cancer, 2011, p 393-410. [2] Mason et al., MRM, 1991, 18, p 71-79. [3] Dardzinski and Sotak. MRM 1994, 32:88-97. [4] Carano et al., MRM 2004, p.542-51. [5] Ungersma et al., MRM 2010, p 1637-47.