A DTI Study to Probe Tumor Microstructure And Its Connection With Hypoxia

Shreyan Majumdar, Mrignayani Kotecha*, William Triplett, Boris Epel, Howard Halpern

Abstract— **Solid tumors have chaotic organization of blood vessels, disruptive nerve paths and muscle fibers that result in a hostile and heterogeneous microenvironment. These tumor regions are often hypoxic and resistant to radiation therapy. The knowledge of partial pressure of oxygen concentration (pO_2), in conjunction with the information about tissue organization, can predict tissue health and may eventually be used in combination with intensity-modulated radiation therapy (IMRT) for targeted destruction of radiation-resistant areas, while sparing healthy tissues. Diffusion tensor imaging (DTI) based parameter fractional anisotropy (FA) can be used to assess organization of tissue microstructure, whereas the pO_2 can be measured using electron paramagnetic resonance oxygen imaging (EPROI). This study is our first step to connect these two important physiological parameters. We calculated FA in fixed fibrosarcoma (FSa) grown in hind leg of nude mice (n = 6) using preclinical 9.4 T MRI. The FA in tumor region (0.34 ± 0.014) was found to be lower when compared to normal surrounding region (0.36 ± 0.013). We hypothesized that the change in FA is directly correlated with the change in oxygen concentration in tumor. We present preliminary *in vivo* results showing a positive correlation (R = 0.85, p = 0.017) between the FA and pO_2 values acquired for MCa4 tumor (n = 1) using DTI and EPROI.**

I. Introduction

Oxygen is crucial for a function of living tissue. Partial oxygen pressure (pO_2) is an important physiological parameter for many diseases, such as cancer, ischemia, cerebrovascular disease and wound healing. Solid tumors have a highly heterogeneous environment, frequently with regions of very low oxygen concentration. These areas with low pO_2 (hypoxic areas) are resistant to the radiation treatment and may require much higher radiation doses for destroying the tumor cells [1]. Thus, the knowledge about the oxygenation levels of tumor tissue is vital when it comes to imparting treatment through radiation.

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Hypoxia can be divided into two broad categories – Chronic and Acute [2]. In growing tumors, the distance between the cells and blood vessels becomes greater than that the oxygen molecule can diffuse through, which results in reduced oxygen supply and consequently, chronic hypoxia. On the other hand, acute hypoxia is caused by fluctuating blood flow in the tumor vessels [2]. In case of chronically hypoxic regions, studies have shown that once hypoxia occurs in tumors, it has led to structural changes in the hypoxic tissue when compared to normoxic tissue [3].

The tumors have abnormal organization of blood vessels and tissue structure that results in heterogeneous perfusion and extravasation, and a hostile microenvironment with increased interstitial pressure [4]. The higher cellularity, tissue disorganization and increased extracellular space all result in a change in tissue organization and lower apparent diffusion coefficients for malignant tumor when compared to normal tissue [5]. This disorganization leads to higher interstitial pressure and chaotic vascular tissue structure as discussed by Jain et al. [4, 6].

Oxygen concentration with high precision (<1 torr) can be measured using the established technique, known as electron paramagnetic resonance oxygen imaging (EPROI) [7, 8]. It has been shown recently that by using EPROI pO_2 information, tumor radiation treatment outcome can be predicted, based on a high or low fraction of image voxels less than 10 torr [7].

Over the past few years, diffusion tensor imaging (DTI) based fractional anisotropy (FA) has been increasingly used to get information about orientation and organization of tissue microstructure in brain tumors [9-12]. However, its use in the case of other tumors is rather limited.

This study is the first step to establish a correlation between these two physiologic parameters, tissue organization and pO_2, using DTI and EPROI at the tissue microstructure level. Our long-term goal is to improve the outcome of radiation therapy by sparing the healthy tissue volume within the tumor using the knowledge obtained from FA and pO_2 images. In the current study, we used either FSa or MCa4 tumor grown in the hind leg of nude mice. We first acquired FA values for fixed FSa tumor (n = 6) and established a statistically significant difference between tumor and normal region based on FA values. We then acquired *in vivo* FA map for MCa4 (n = 1) and registered it with pO_2 map acquired using EPROI. We obtained the Pearson correlation coefficient and p-value for *in vivo* FA and pO_2.

II. THEORY

A. Diffusion Tensor Imaging (DTI): Water is the primary source of MRI signal in tissues. In tissue, water motion is not entirely random (Brownian) and is restricted by cellular membranes, intracellular organelles and macromolecules. In MRI experiments, diffusion coefficient, D, is measured by using a pair of gradient pulses in the standard spin echo experiment. The signal strength in this case is given by

$$S = S_0 e^{-bD}, where\ b = \gamma^2 G^2 \delta^2 \left(\Delta - \frac{\delta}{3}\right) \quad (1)$$

where γ is gyromagnetic ratio of water protons, G is gradient strength, δ is gradient duration and Δ is delay between the pair of gradients. Fitting the echo intensity with exponential decay as a function of b-values gives an estimation of diffusion coefficient, D. This parameter is commonly referred as Apparent Diffusion Coefficient (ADC).

Typically, biological tissues are heterogeneous and therefore single ADC value is insufficient to describe the tissue microstructure. In such case, diffusion is anisotropic and not scalar, and diffusion tensor imaging (DTI) can be used to get information on directionality of water diffusion. A second-order diffusion tensor or a 3x3 covariance matrix represents the water diffusion in tissue. The diffusion tensor may be visualized using an ellipsoid where its eigenvectors define the principal axis directions and its eigenvalues (λ_1, λ_2, and λ_3) define the radii. The movement of water molecule can be described by two metrics, mean diffusivity (MD) and fractional anisotropy (FA), which represents the magnitude and directionality of water diffusion respectively [11, 13]. The FA is given by [14, 15]:

$$FA = \sqrt{\frac{3}{2} \times \frac{(\lambda_1 - MD)^2 + (\lambda_2 - MD)^2 + (\lambda_3 - MD)^2}{(\lambda_1^2 + \lambda_2^2 + \lambda_3^2)}} \quad (2)$$

Diffusion is considered isotropic when eigenvalues are equal in all directions and FA is 0 in this case. The highest value of FA equal to 1 represents the unidirectional diffusion.

B. Electron Paramagnetic Resonance Oxygen Imaging (EPROI): EPROI is a minimally invasive technique to derive absolute value of pO_2 [16]. The Smoluchowski diffusion equation predicts a linear relationship between pO_2 and relaxation rates (R_1 or R_2) of the paramagnetic spin probe injected into an animal that is validated for multiple radicals. Recent advances in EPROI use the spin-lattice relaxation dependence on oxygen concentration using the equation:

$$R_1 = A*\{pO_2\}+Rx \quad (3)$$

where R_1 is spin-lattice relaxation rate of injected spin-probe, $\{pO_2\}$ is absolute pO_2 concentration in the tissue and Rx is oxygen independent relaxation caused by other factors such as temperature, salinity, and viscosity. The relaxation Rx caused by these other factors besides pO_2 is weak in comparison to the oxygen proportionality coefficient A and is well known. This allows a direct measure of pO_2 with a precision exceeding 1 torr and is a unique feature of EPROI. The hypoxia is typically defined as a pO_2 values below 10 torr. The pO_2 statistics is typically based on hypoxic fraction below 10 torr, HF10, criterion.

III. MATERIALS AND METHOD

A. Tumor Preparation $5x10^5$ murine tumor cells (FSa or MCa4) were injected intramuscularly in the hind leg of a 6 to 8 week old C3H mice. The tumor grew to 0.5 - 1 cm^3 within 1-2 weeks. For the *in vivo* experiment, the tumor was immobilized using a vinyl polysiloxane dental mold to encompass around half of the tumor bearing leg. MRI and EPROI experiments were performed sequentially under isoflurane anesthesia. For experiments with fixed tumor, the animals were euthanized and fixed using 10% formalin.

***B. DTI Experiments with Fixed Tumor*:** All fixed tumor MRI experiments were performed using a 9.4 T preclinical Agilent MRI scanner equipped with 60 mm/ 115 mm rf coil/gradient pair at the University of Illinois at Chicago (UIC). The experimental parameters were: pulse protocol = spin echo DTI (semsdw), TE/TR = 30 ms / 4 s, number of slices = 20, slice thickness = 0.75 mm, b-values = 0, 1500 s/mm^2, δ = 5 ms, Δ = 20 ms, FOV = 3.0 x 3.0 cm, matrix size = 128 x 128, number of different gradient directions = 6.

***C. In Vivo DTI experiment*:** The *in vivo* DTI experiment was performed using a 9.4 T preclinical Bruker MRI scanner with a custom-built 30 mm rf coil at the University of Chicago. The diffusion weighted spin echo sequence was used with 6 different gradient directions. The other experimental parameters were: TE/TR = 19 ms / 2500 ms, slice thickness = 0.75 mm, number of slices = 3, δ = 7 ms, Δ = 14 ms, FOV = 2.56 cm x 2.56 cm, matrix size = 128 x 128, and b-values = 0, 2500 s/mm^2, total experimental time ~ 39 min.

***D.* EPROI Experiment:** *In vivo* EPROI experiment was performed immediately following DTI experiment on the same animal. We used pulse EPR inversion recovery methodology for pO_2 image acquisition [8]. 208 equal solid angle projections were acquired with maximum gradient of 15 mT/m and a isotropic field of view of 4.24 cm. Images were reconstructed using filtered back projection algorithm. OX063 spin probe was injected IV into animal 0.56 mmol/kg followed by infusion at 0.78 mmol/kg/hr during imaging time. Image was acquired in 10 minutes and had 1.5 mm spatial and 1 torr pO_2 resolution.

***E. Data Analysis and Image Registration*:** The FA maps were calculated using IDL virtual machine based MRI analysis software (MAS) developed at the University of Florida by Prof. T. Mareci's research group [17]. The mean FA for fixed tumor and surrounding normal region was calculated using region of interest (ROIs) from a single middle slice based on diffusion maps. An example of MD and FA map for tumor and normal region is shown in Figure 1. The *in vivo* FA images were registered with oxygen images using a custom built Matlab program and statistics is based on the number of slices acquired.

IV. RESULTS

Figure 1 shows an example of MD and FA maps obtained using a fixed tumor tissue. It is known that solid

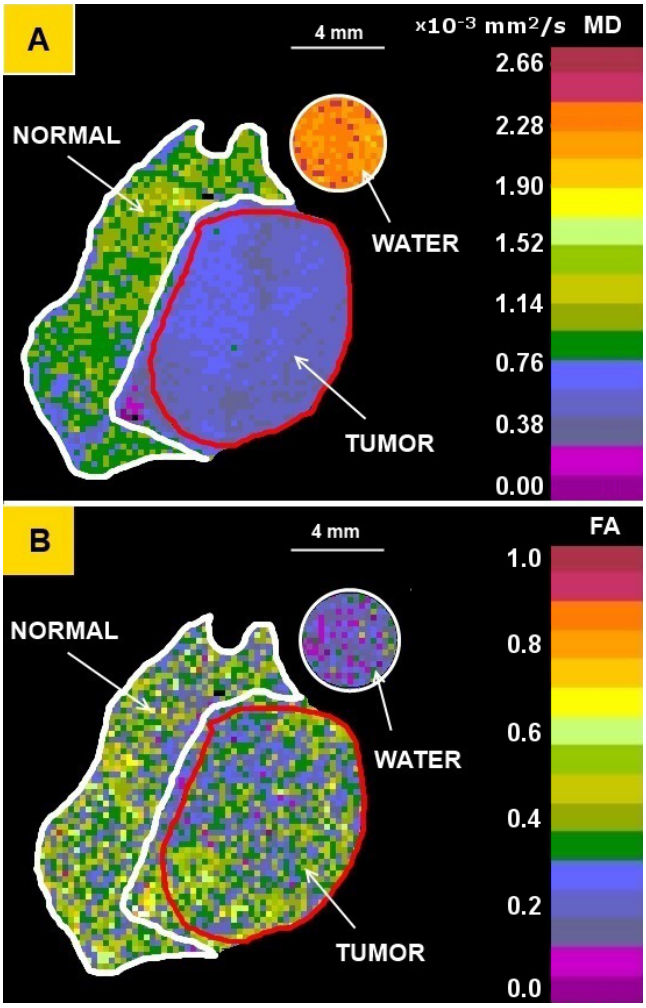

Figure 1: Representative example of (A) mean diffusivity (MD) and (B) fractional anisotropy (FA) maps from an axial slice in a fixed tumor and surrounding normal region.

tumors have lower apparent diffusion coefficient (ADC) than surrounding normal region and our data is consistent with the published literature [18]. The mean diffusivity in tumor region {MD_{tumor} = 0.61 (± 0.1) x 10^{-3} mm^2/s} was found to be significantly lower than the surrounding normal region {MD_{normal} = 0.93 (± 0.2) x 10^{-3} mm^2/s}.

The mean value of FA for tumor 0.34 (±0.01) was also found to be smaller than FA of normal regions 0.36 (±0.01) as listed in Table 1. The FA values obtained are consistent with the reported literature values of FA of about 0.2 – 0.4 in muscle [19]. However, the difference between normal and tumor FA is not so visually distinguishable. Note that fixation might have an impact on FA of tumor and normal regions. It is known that fixing introduces cross-linking in the tissues and long time fixing have effect of altering tissue properties [20]

Figure 2 shows the first of the kind pO_2 and FA map registered for MCa4 tumor. In the tumor region, lower FA ≤ 0.5 correlated with hypoxic region (~ 5 torr) visually. The histogram shown in Figure 2(C) and 2(D) confirms the lower FA for hypoxic tumor region. Table 1 lists mean FA and pO_2 for tumor and normal region *in vivo*. The mean FA in tumor 0.53 (± 0.01) is lower when compared with mean FA in

Table 1: FA and pO_2 in tumor and normal region

Tumor type		Tumor	Normal
		Mean (± SEM)	Mean (± SEM)
FSa (n=6)	FA	0.34 (± 0.014)	0.36 (± 0.013)
MCa4 (n =1)	pO_2 (torr)	11.07 (± 0.16)	27.4 (± 0.3)
	FA	0.53 (± 0.01)	0.66 (± 0.01)

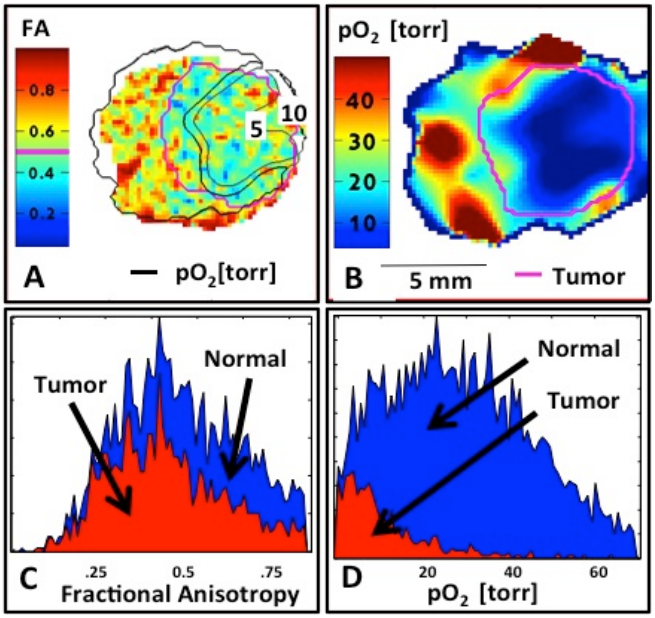

Figure 2: (A) Fractional anisotropy (FA) and (B) oxygen images from an axial slice of a murine leg with MCa4 tumor *in vivo*. Color bar shows FA scale in the range of 0 – 1 and pO_2 scale in the range of 0 - 50 torr. (C) and (D) shows histogram for FA and pO_2 values from the whole image (blue) and from the tumor volume only (red). The cyan contour on both FA and pO2 images shows the digitized tumor contour. On the FA image slice, contours of 10 and 5 torr pO_2 from EPROI are shown. Note that only 3 slices were acquired for DTI experiments thus showing smaller amount of normal tissue for FA histogram and statistics.

normal region 0.66 (± 0.01). The mean pO_2 in tumor was also found to be lower 11.7 (± 0.16) torr when compared to normal surrounding regions with pO_2 values of 27.4 (± 0.3) torr. The FA difference between normal and tumor region *in vivo* is larger in comparison with fixed tissues, that might be

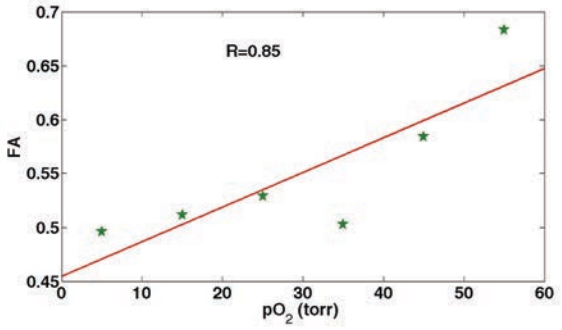

Figure 3: Correlation between mean pO_2 and FA values within the tumor volume obtained from *in vivo* (n = 1) data. The red line is provided for eye guidance. The calculated Pearson correlation coefficient R is 0.85 with p-value 0.017.

the because of different tumor or effect of fixation.

Figure 3 shows a plot of FA vs voxel pO_2 within the tumor volume. The Pearson correlation coefficient R = 0.85 with p = 0.017 shows a highly significant correlation between FA and pO_2 in this data set.

V. Conclusion

We present preliminary results to establish a connection between tissue organization and oxygen concentration in tumors. Our long-term goal of this project is to improve the efficacy of radiation treatment by sparing the healthy tissues from unnecessary excessive radiation. We expect that the knowledge of pO_2 in conjunction with tissue organization will be important to improve radiation treatment in future.

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