Tumor Microenvironment session

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Imaging Tumor Hypoxia

Target audience – Imaging scientists/radiologists, oncologists, physiologists, and scientists interested in tumor oxygenation.

Highlights

1. Tumor hypoxia influences angiogenesis, metastasis, and response to therapy. Therefore, the ability to identify hypoxia could allow stratification for optimized therapy.
2. Various MRI methods have been developed to quantify tumor hypoxia and pO_2 or provide pertinent surrogate biomarkers.
3. This presentation will consider virtues and shortcomings of diverse techniques in terms of ease of implementation and nature of observations (spatial resolution, precision, dynamics, and need for exogenous reporter agents).
4. Examples will be drawn from pre-clinical studies of mice and rats, as well as recent translational observations in human patients.

Purpose: There is increasing evidence for the importance of tumor oxygenation in the development, progression, and response to therapy. Consequently, many techniques have been developed to assess tumor oxygenation, as reviewed extensively [1-3]. Methods may provide a qualitative impression of oxygenation status or rigorous quantitation. Techniques vary in spatial and temporal resolution and the ability to assess dynamic changes. Some exploit endogenous molecules or physical characteristics, while many apply reporter molecules to interrogate oxygen tension (pO_2). This tutorial will focus on magnetic resonance approaches, but place them in the context of competing modalities.

It has long been appreciated that hypoxic tumor cells are relatively resistant to radiotherapy. Indeed, a threefold increase in radio resistance may occur when cells are irradiated under hypoxic conditions compared with $pO_2 > 15$ torr for a single radiation dose. However, modeling indicates that the proportion of cells in the range 0 - 20 torr may be most significant in terms of surviving a course of fractionated radiotherapy [4]. Thus, the ability to measure pO_2 non-invasively, and repeatedly, with respect to acute or chronic interventions becomes increasingly important. Patients could be stratified according to baseline hypoxia to receive adjuvant interventions designed to modulate pO_2. Tumors, which do not respond to such interventions, may be ideal candidates for hypoxia selective cytotoxins (*e.g.*, tirapazamine or TH-304) [5], more intense therapy as facilitated by IMRT (Intensity Modulated Radiation Therapy) or heavy ions such as proton or carbon beam [6]. Noting that any therapy and intervention may have side effects or simply add to clinical costs, it is vital that efficacy be established and therapy be optimized for an individual patient. Whether initially hypoxic regions of a tumor can be modified to become better oxygenated has long been considered a key to improving outcome of irradiation. However, many attempts to improve therapeutic outcome by manipulation of tumor oxygenation have shown only modest success in the clinic, and it is thought that lack of success may have resulted from inability to identify those patients, who would benefit from adjuvant

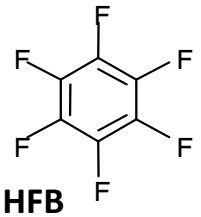
interventions [7]. While pO_2 determinations could be of great clinical value, they are also vital to many laboratory investigations of new drugs and studies of tumor development.

Methodologies

Oximetry: pO_2 may be measured directly using physical interactions between oxygen and reporter molecules. The most popular quantitative approach has exploited the oxygen-dependant ^{19}F NMR spin lattice relaxation rate ($R_1=1/T_1$) of perfluorocarbons (PFCs). A linear dependence $R_1= a + bpO_2$ is observed due to the ideal gas-liquid interaction of paramagnetic molecular oxygen (O_2) dissolving in PFC. PFCs essentially act as molecular amplifiers, since the solubility of oxygen is greater than in water, but thermodynamics require that the pO_2 in the PFC rapidly equilibrates with the surrounding medium. Importantly, ions do not enter the hydrophobic PFC phase, and thus, do not affect the bulk relaxation. The R_1 sensitivity of individual perfluorocarbon resonances varies widely and depends on the intrinsic anoxic relaxation rate, the solubility of oxygen, and the ability of the oxygen molecule to approach molecular moieties. Early studies focused on perfluorotributylamine (PFTB) and perfluorooctylbromide (PFOB) [8] and these were widely exploited for spectroscopy. However, multiple resonances can lead to chemical shift artifacts in images, requiring more sophisticated imaging approaches, which often sacrifice signal. Recent work has favored perfluoro-15-crown-5-ether (15C5) and hexafluorobenzene (HFB), which each exhibit a single ^{19}F resonance, hence maximizing SNR [9].

PFCs are extremely hydrophobic but may be formulated as biocompatible emulsions for IV administration. Shortly after administration, PFC in the blood provides measurements of vascular pO_2 [10], but clearance occurs within 1 to 2 days leading to extensive accumulation in the liver, spleen, and bone marrow, providing unique insight into these organs [11]. Limited material does accumulate in other organs and oximetry has been reported with respect to myocardial ischemia [12]. Accumulation in tumors occurs predominantly in regions of greater perfusion, often tumor periphery, potentially biasing measurements [13]. Some PFCs show extended tissue retention allowing chronic studies during tumor development; progressive tumor hypoxiation has been observed over extended periods of many days [13, 14].

To avoid reticuloendothelial uptake (potential hepatomegaly) and bias towards well perfused tumor regions, we favor direct intratumoral (IT) injection of neat PFC allowing any region of interest to be interrogated immediately. Use of a fine needle ensures minimal tissue damage and provides measurements closely analogous to electrodes or fiber optic probes [15]. HFB has many virtues as a pO_2 reporter [16]. Typically 50 – 100 µl are introduced across the tumor to ensure that multiple regions are sampled. HFB has a single narrow ^{19}F NMR signal and the spin lattice relaxation rate is highly sensitive to changes in pO_2, yet minimally responsive to temperature. Recognizing that tumors are heterogeneous and that pO_2 may fluctuate, we developed a procedure [*FREDOM* (Fluorocarbon Relaxometry using Echo planar imaging for Dynamic Oxygen Mapping)], which allows repeated quantitative maps of regional pO_2 to be achieved with multiple individual locations (50-150 voxels with 1.25 mm in plane resolution) simultaneously in 6.5 mins with a precision of 1-3 torr, when pO_2 is in the range 0-15 torr [15]. At 37 °C and 4.7 T: pO_2 (torr) = $(R1(s^{-1}) -0.0835)/0.001876$, so that T_1 reaches 12 s under anoxic conditions. To avoid excessive experimental acquisition time we favor pulse burst saturation recovery (PBSR) echo planar imaging (EPI) relaxometry. Traditional T_1 measurement sequences acquire data with delays in monotonic order, whereas we alternate longer and shorter delays to minimize any systematic errors, which would be introduced, if the signal amplitude varies during the measurement (ARDVARC (Alternated Relaxation Delays with Variable Acquisitions to Reduce Clearance effects) [15]. Gallez *et al.* have accelerated the acquisition to provide pO_2 maps within 90 s based on a Look-Locker (SNAP-IR) approach [17].



HFB

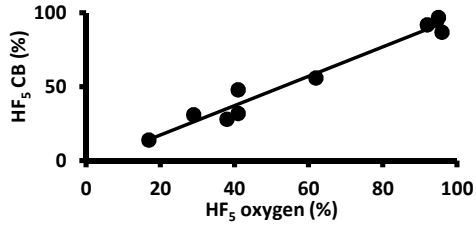
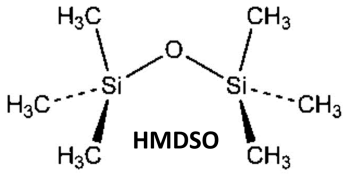


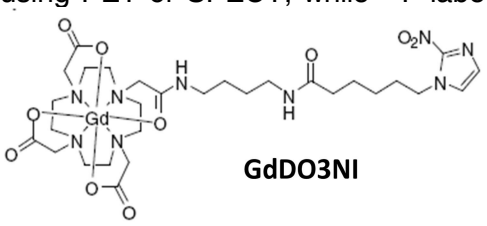
Figure 1. Comparison of the response to oxygen (O_2) and carbogen (CB) for 9 Dunning prostate R3327-AT1 tumors. Correlation between residual hypoxic fraction (pO_2 < 5 Torr) in individual tumors, while breathing O_2 or CB. Modified from [51].

The most powerful aspect of *FREDOM* is the ability to follow the fate of individual tissue regions of interest (voxels) with respect to interventions. Most extensive investigations have focused on the response to respiratory challenge, often comparing the effects of oxygen (O_2) versus carbogen (CB) gas breathing (*e.g.*, Fig. 1) [2, 18-20]. Most significantly, it has been shown that the ability to modulate pO_2, as assessed using *FREDOM* correlated with tumor growth delay accompanying single high dose irradiation [21, 22]. In some tumor types, there is a strong correlation between mean pO_2 and hypoxic fraction, though this is not always the case. Following administration of the vascular disrupting agent (VDA) Combretastatin (CA4P) rapid hypoxiation of rat breast tumors was observed within 30 minutes followed by differential localized recovery 24 h later [23]. Arsenic trioxide (ATO) has been described as a VDA [24], but Diepart *et al.* unexpectedly found increased pO_2 within 30-90 minutes (depending on tumor type) of a relatively low dose (5 mg/kg) and demonstrated that this followed mitochondrial impairment [25]. For both CA4P and ATO the temporal evolution of tumor oxygenation, allowed timing of radiation to be optimized to achieve enhanced tumor control [25, 26]. We believe that quantitative PFC oximetry provides a valuable pre-clinical tool, though ultimately it may be most appropriate to calibrate non-invasive observations such as BOLD and MOXI approaches described below. We recognize that ^{19}F remains quite esoteric on clinical scanners and thus proton MRI methods appear preferable.

We recently demonstrated a proton analog of HFB, specifically hexamethyldisiloxane (HMDSO). Like HFB, HMDSO is highly hydrophobic giving high gas solubility, and hence strong R_1 response to changes in pO_2. Symmetry provides a single proton resonance (δ = 0 ppm), which is well removed from water and fat and we have achieved dynamic maps of tumor oxygenation with respect to hyperoxic gas breathing challenge [27]. As opposed to direct pO_2 measurements, various studies have indicated correlation between DCE parameters (K_{Trans} and/or V_e) and tumor hypoxia, as would be expected based on perfusion dependence [28-30].



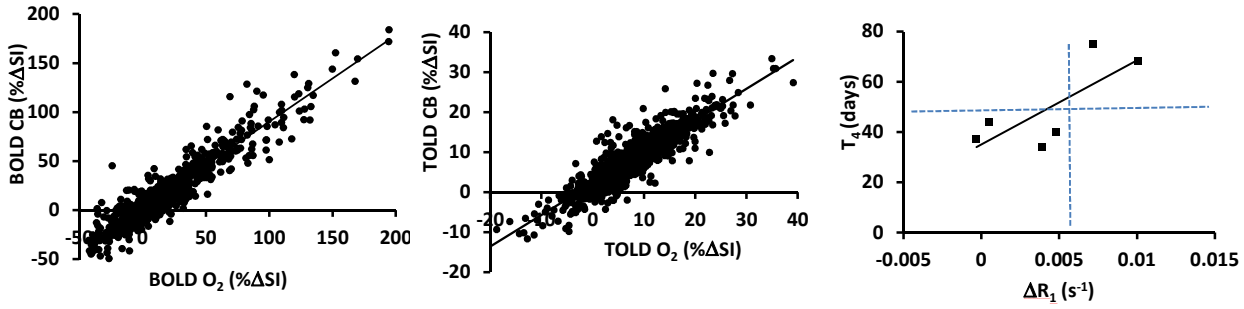
Hypoxia An alternative to quantitative oximetry is direct assessment of hypoxia. Nitroimidazoles were initially designed as chemical modifiers of cancer treatment, specifically becoming trapped in hypoxic tissues and enhancing radiation sensitivity [31]. Specific molecular modifications have produced reporter molecules to reveal hypoxia (*e.g.*, pimonidazole, EF5, CCI-103F, galactopyranoside IAZA) [2]. Following IV infusion, these agents become reduced in tissues and in the absence of oxygen are trapped. However, in the presence of oxygen they are reoxidized and ultimately clear from the body. Histological assessment of the distribution of these agents (*e.g.*, EF5 and pimonidazole) provides microscopic indications of local hypoxia. Many molecular structures have been proposed over the past 25 years and incorporation of radionuclides has facilitated non-invasive investigations using PET or SPECT, while ^{19}F labels permit NMR spectroscopy [2, 32]. Several ^{19}F NMR hypoxia agents have been tested, *e.g.*, hexafluoromisonidazole (CCI-103F), EF5, NLTQ-1, SR-4554, and Ro 07-0741). Variants have also been generated as ^{1}H MR reporters both for spectroscopy [33] and imaging [34]. Indeed the retention of GdDO3NI matched the pattern of oxygenation expected in



Dunning prostate R3327-AT1 tumors based on extensive previous ^{19}F oximetry imaging.

Assessment of hypoxia is predicated on uptake and trapping of the reporter, assessed as the relative signal at various times (retention index) or based on the relative signals from tumor and surrounding control tissues. Weak ^{19}F signals generally restrict measurements to a global value across the whole tumor. Trapping may also depend on expression of nitroreductases and be influenced by glutathione [32]. Likewise, tumor perfusion may influence access of the agents to tumor tissue, particularly poorly perfused regions, which are expected to be hypoxic. Indeed, uptake of hypoxia reporters following administration of vascular disrupting agents, did not match hypoxia, presumably because access was hindered to the very regions which became hypoxic [35]. While pO_2 reporters such as HFB allow rapid repeat measurements revealing acute dynamic changes accompanying interventions and natural fluctuations [36], the hypoxia agents generally provided a single time point only, though dynamic variations in hypoxia have been observed, even in biopsy specimens, by applying pairs of hypoxia reporters in a pulse chase fashion [37].

Non-invasive Oxygen Enhanced MRI: Imaging per se is non-invasive and it would be particularly attractive to develop oximetry methods based on properties of endogenous molecules, rather than requiring administration of reporter agents. Lactate concentration has been associated with hypoxia as consistent with impaired oxidative phosphorylation and accelerated glycolysis, though many factors may influence this phenotype [38]. BOLD (Blood Oxygen level Dependant) contrast ^{1}H MRI is directly sensitive to deoxyhemoglobin concentration and forms the basis of so-called functional MRI (fMRI), as used to reveal neuronal activation. Extensive studies have demonstrated BOLD effects in tumors most commonly in response to a hyperoxic gas breathing challenge (*e.g.*, Fig. 2A).



A **B** **C**

Figure 2. Oxygen enhanced MRI of Dunning prostate R3327-AT1 tumor. Correlations observed between response to oxygen and carbogen challenge for individual voxels in a representative tumor based on A) T_2W signal response (BOLD), B) T_1W signal response (TOLD). C). Correlation between time for tumor to quadruple is size (T_4) and ΔR_1 for those tumors irradiated during oxygen breathing (n=6). Modified from [51].

Several studies have demonstrated correlations between BOLD response and changes in pO_2 in various tumor types [39-41]. The dependence is often non-linear, but distinct trends have been observed. Measuring BOLD response to oxygen change is readily implemented for human subjects and studies have been reported at various disease sites including breast [42], cervix [43], prostate [44, 45] and brain [46]. Early reports examined changes in T_2^*-weighted signal intensity (ΔSI), but it was rapidly appreciated that ΔSI is also subject to flow effects and the concept FLOOD (Flow and Oxygen Dependant) contrast was suggested [47]. Quantitative measurement of R_2^* should mitigate flow effects. Rodrigues *et al*. demonstrated that tumors with fast R_2^* and large ΔR_2^* showed enhanced response to radiation when mice breathed CB, whereas RIF1 tumors showed much smaller effects and no benefit from CB breathing [48]. In human prostate tumors trends have been noted between baseline R_2^* and pO_2 or hypoxia [44, 49], without the need for a gas challenge. However, it must be recognized that BOLD depends on vascular deoxyhemoglobin and is therefore influenced by vascular extent, volume, flow and hematocrit.

Indeed, an attempt to calibrate BOLD in terms of absolute pO_2 based on a hypoxic endpoint (breathing nitrogen) in rat tumors generated a seemingly inconsistent result with R_2* decreasing upon death, likely due to blood (*viz*. deoxyhemoglobin) leaving the tumor as a consequence of reduced systemic blood pressure [47].

It has been suggested that tissue water R_1 should more closely match changes in pO_2 based on paramagnetic properties of dissolved O_2. This has been adopted as the TOLD (Tissue Oxygen Level Dependant) concept [50]. Several studies have now reported T_1 response to interventions such as oxygen breathing challenge (Fig. 2B) [51-55]. Logically, one might expect BOLD changes to be followed by TOLD response based on progressive vascular oxygenation followed by diffusion of oxygen into tissues generating elevated pO_2 [51]. Several studies have now shown close correlation between BOLD and TOLD responses, but some tumor types show distinct mismatch [51, 55 54]. It must be remembered that deoxyhemoglobin also has a small effect on T_1, while $[O_2]$ can affect T_2* and therefore under specific conditions one or other effect may dominate based on vascular extent and perfusion. Notably two studies have demonstrated correlation between tumor growth delay following high dose irradiation of tumors and TOLD response to oxygen breathing challenge, whereas BOLD response was not correlated in these specific tumor types [51, 56]. Specifically, a large TOLD response indicated tumors which would benefit from rats breathing oxygen during irradiation.

Recognizing the greater solubility of oxygen in lipids as compared with water Gallez *et al*. recently proposed MOBILE (Mapping of Oxygen By Imaging Lipids relaxation Enhancement) [57]. They specifically showed that changes in lipid relaxation were greater than water, about 2 and 11 fold greater for the resonances at 1.2 and 4 ppm respectively. Observations were reported in mice, with respect to ischemia, liver steatosis, and tumors and also in human volunteers.

Recently, Zhang *et al.* [58] demonstrated that a multi parametric analysis of tumor water signal could directly provide estimates of pO_2. Specifically, biexponential analysis of IVIM (Intra Voxel Incoherent Motion) allowed estimation of tumor vascular volume; then in combination with R_1 and biexponential analysis of CPMG-based R_2, yielding the extravascular component, an accurate measurement of pO_2 was achieved, as validated using ^{19}F MRI of PFC.

Conclusion Notably, BOLD and TOLD MRI have been successfully applied in studies of normal human volunteers and patients enrolled in trials with respect to cancer in various disease sites. It remains to be seen which parameter is most useful in identifying patients to characterize tumors for optimal therapy. The enhanced oxygen sensitivity of MOBILE and quantitative estimates provided by MOXI are very promising though their general applicability remain to be evaluated. Quantitative ^{19}F oximetry remains a valuable pre-clinical tool and continues to serve as a validation for less invasive approaches. If ^{19}F MRI becomes more readily available the reporter molecule approaches may find expanded applications. The capabilities discussed in this tutorial will soon be available as part of a book chapter [59].

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