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Developing oxygen-enhanced magnetic resonance imaging as a prognostic biomarker of radiation response ☆

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ABSTRACT

Oxygen-Enhanced Magnetic Resonance Imaging (OE-MRI) techniques were evaluated as potential non-invasive predictive biomarkers of radiation response. Semi quantitative blood-oxygen level dependent (BOLD) and tissue oxygen level dependent (TOLD) contrast, and quantitative responses of relaxation rates (ΔR_1 and ΔR_2^*) to an oxygen breathing challenge during hypofractionated radiotherapy were applied. OE-MRI was performed on subcutaneous Dunning R3327-AT1 rat prostate tumors ($n = 25$) at 4.7 T prior to each irradiation ($2F \times 15$ Gy) to the gross tumor volume. Response to radiation, while inhaling air or oxygen, was assessed by tumor growth delay measured up to four times the initial irradiated tumor volume (VQT). Radiation-induced hypoxia changes were confirmed using a double hypoxia marker assay.

Inhaling oxygen during hypofractionated radiotherapy significantly improved radiation response. A correlation was observed between the difference in the 2nd and 1st ΔR_1 ($\Delta\Delta R_1$) and VQT for air breathing rats. The TOLD response before the 2nd fraction showed a moderate correlation with VQT for oxygen breathing rats. The correlations indicate useful prognostic factors to predict tumor response to hypofractionation and could readily be applied for patient stratification and personalized radiotherapy treatment planning.

Introduction

Hypoxia is increasingly recognized to play a fundamental role in aggressiveness and therapeutic resistance in many tumors including prostate [1–5]. Hypoxia has been associated with radioresistance in cells [6], pre-clinical animal studies [7–12] and human patients [3,5,13]. However, the meta-analysis of Overgaard [14] indicated that interventions to overcome hypoxia provided only marginal benefit and it was concluded that the lack of efficacy was likely related to the inability to identify which patients would benefit. Consequently, there is a substantial effort to develop non-invasive measurements of the dynamics of tumor oxygenation as potential biomarkers for patient stratification [2,15,16].

Robust evidence for hypoxia in human tumors has been established at multiple disease sites using the Eppendorf Histograph electrode system [1,4,5,17–20]. This has also been applied extensively in pre-clinical studies, but is highly invasive, technically challenging and no longer commercially available. Analogous measurements of tumor pO_2 and hypoxic fractions have been achieved by direct intra tumoral administration of reporter molecules for ^{19}F [8,9,21] and ^{1}H MRI [22], and ESR [11,23,24]. These have the distinct benefit of allowing dynamic response to interventions to be assessed non-invasively [8,9,11,22–25]. To avoid violating tumor integrity, reporter molecules may also be delivered intravenously [10,26], but such measurements invariably bias results toward better perfused and likely less hypoxic regions. The need for reporter molecules complicates potential translation to the clinic.

Hypoxia may be directly observed using nuclear medicine reporters, typically ^{18}F labeled nitroimidazoles [15,16,27], but the associated radioactivity makes them expensive and assessment of dynamic modulation of hypoxia is generally not practical. Analogous use of immunochemistry of nitroimidazole trapping has allowed pulse chase evaluation of hypoxia modulation, but requires biopsy [28].

Oxygen enhanced MRI has been suggested as a potential alternative approach, since it is entirely non-invasive and can be readily added to routine clinical MRI, which is increasingly applied to radiation planning and execution [29]. The tissue water proton

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apparent transverse relaxation rate (R_2^*) is strongly influenced by the concentration of deoxyhemoglobin, which is paramagnetic [2,28]. This provides blood oxygen level dependent (BOLD) contrast, which is the basis of fMRI used in studies of neuronal activation. R_2^* is influenced by conversion of deoxy- to oxyhemoglobin, but is also subject to alteration in flow, hematocrit, and vascular volume, as described by the so-called FLOOD effect [30]. Meanwhile, the spin lattice relaxation rate (R_1) is directly sensitive to the concentration of free oxygen molecules and hence pO_2. This is the basis of tissue oxygen level dependent (TOLD) contrast [31]. Several investigations have examined correlations between BOLD and TOLD based on semi quantitative changes in signal intensity or quantitative relaxation maps. Notably, studies in human tumor xenografts in mice [32], as well as syngeneic tumors in rats [33,34] and rabbits [35]. The two approaches have also been assessed in humans including volunteer patients [36–38].

Several studies have examined correlations of BOLD with invasive oximetry in pre-clinical studies based on polarographic oxygen electrodes, fluorescent quenched fiber optic probes and ^{19}F MRI [39–41]. Sometimes a strong direct correlation has been observed, while other studies indicated nonlinear correlative trends. Notably, a large BOLD response to a hyperoxic gas breathing challenge was associated with elimination of hypoxia in 13762NF rat breast tumors [41]. A recent report indicated that syngeneic rat prostate tumors could be stratified in terms of radiation response based on TOLD MRI responses to an oxygen breathing challenge before a single dose of radiotherapy [33].

New hypofractionated treatment approaches are gaining popularity for several reasons: (i) fewer treatment sessions are convenient to patients and physicians; (ii) precise treatment plans may be developed for each irradiation; (iii) recent clinical trials are showing enhanced outcome [42]. However, it is thought that radiation response is more influenced by hypoxia, especially when large single- or multi-fraction dose regimens typical of stereotactic body radiotherapy (SBRT) are implemented, since tumor reoxygenation is minor compared to traditional conventional fractionated radiation therapy (CFRT) [43].

Noting the importance of hypoxia and desire for a robust non-invasive approach to assess tumor hypoxia and oxygen dynamics prompted us to explore OE-MRI with respect to a hypofractionated radiation regimen.

Materials and methods

Investigations were approved by the Institutional Animal Care and Use Committee. The experimental timeline for separate groups of tumors is shown in Table 1. Additional experimental details are provided in Supplementary Materials.

Dunning R3327-AT1 prostate tumors were surgically implanted subcutaneously in the flank of 25 adult male syngeneic Copenhagen rats [9]. The AT1 is a well-characterized anaplastic prostate tumor often used for radiobiological studies [9,21,33,44–46]. Tumors were used for OE-MRI around 19 days after implantation, when they reached a size in the range 0.7–2.1 cm^3. Animals were divided into four groups: unirradiated "Control" (Group 1, $n = 4$), irradiated while inhaling "Air" (Group 2, $n = 9$), irradiated while inhaling "Oxygen" (Group 3, $n = 9$) and immunohistological correlates (Group 4, $n = 3$).

Oxygen-enhanced magnetic resonance imaging

Anesthetized rats were provided with a warming pad to maintain body temperature, placed in a 4.7 T MR scanner and physiological parameters recorded using an MR-compatible monitoring and gating system. Baseline R_1 measurements of the tissue water proton signal were obtained with a 2-D multi-slice spin-echo (SEMS) sequence, while the animals breathed air (1 dm^3/min with 1.5–2.0% isoflurane) and at the end of the oxygen breathing challenge. Interleaved dynamic blood-oxygenation level dependent (BOLD or R_2^*) and tissue-oxygenation level dependent (TOLD or T_1-weighted) measurements were performed for about 10 minutes for baseline air and during a hyperoxic oxygen breathing challenge (1 dm^3/min O_2 up to 10 minutes). BOLD acquisition used a 2-D multi-slice spoiled gradient-echo with multi-echo (MGEMS) sequence.

Radiation therapy

Tumors were irradiated about 24 hours after OE-MRI experiments. Prior to, and during radiotherapy, the anesthetized animals inhaled either Air (Group 2, $n = 9$) or Oxygen (Group 3, $n = 9$) for at least 15 minutes. Unirradiated tumors ($n = 4$) provided controls. Radiation was applied to the gross tumor volume (GTV) with orthovoltage x-rays at 15 Gy using image-guided radiation therapy with a small animal x-ray irradiator. OE-MRI and irradiation were repeated one week later. Tumor growth was measured weekly until tumors reached 10% body weight or 90 days to assess the response to radiation. Tumor growth delay was determined by the time required for the tumors to reach two (volume doubling time, VDT) and four times (volume quadrupling time, VQT) the initial irradiated tumor volume using simple linear interpolation. Three additional tumors (Group 4) were examined to assess reoxygenation after the first fraction based on immunohistochemistry.

Immunohistochemistry

A double hypoxia marker approach [28,47] was used to verify tumor reoxygenation. Immediately after OE-MRI, three tumor bearing rats, while breathing oxygen, were injected intravenously with pimonidazole as a baseline tumor hypoxia marker. About 24 hours later two of the tumors were irradiated with 15 Gy, while the animal was breathing oxygen. The third tumor served as a control. Three days later a second tumor hypoxia marker, CCI-103F, was injected intraperitoneally, while the rats were breathing oxygen. Two hours later, the rats were sacrificed and tumor tissue harvested.

OE-MRI data processing and analysis

Using in-house algorithms developed in Matlab, voxel-by-voxel %ΔSI in BOLD and TOLD responses with respect to inhaling oxygen was calculated from the whole tumor region-of-interest. BOLD images were selected at a single echo time (TE = 20 ms) for analysis. Voxel-by-voxel R_2^* maps were generated from BOLD images by fitting the multi-echo data to the echo time (TE) in a nonlinear least squares equation and quantitative ΔR_2^* values were calculated. Likewise, R_1 with respect to the repetition times (TR). A log-rank test Kaplan–Meier analysis was used to compare tumor growth for Air, Oxygen and Control groups.

Results

Oxygen-enhanced magnetic resonance imaging

Tumors showed considerable heterogeneity in terms of baseline R_2^* and R_1, as well as responses to oxygen challenge (semi

Table 1
Chronological scheme of measurements and irradiation.

Day	0	19	19	20	23	26	27	Termination
Procedure	Implant	OE-MRI[a]	Pimonidazole	IR 15 Gy	CCI-103F	OE-MRI	IR 15 Gy	VQT, 10%BW loss 90 days
Group 1 No IR	n = 4							√
Group 2 2 × 15 Gy Air	n = 9	Air → O_2 R_1, IBT, R_1		Air		Air → O_2 R_1, IBT, R_1	Air	√
Group 3 2 × 15 Gy O_2	n = 9	Air → O_2 R_1, IBT, R_1		O_2		Air → O_2 R_1, IBT, R_1	O_2	√
Group 4 IHC	n = 3	Air → O_2 R_1, IBT, R_1	O_2	O_2 (n = 2)	OE-MRI, O_2, sacrifice			

[a] R_1, IBT, R_1 indicates baseline R_1 map followed by interleaved R_2^* maps and T_1-weighted images during transition to oxygen followed by final R_1 map with O_2 breathing.

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