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Electron paramagnetic resonance (EPR) oxygen image hypoxic fraction plus radiation dose strongly correlates with tumor cure in FSa fibrosarcomas

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Abstract

Purpose—Tumor hypoxia has long been known to produce resistance to radiation. In this study, electron paramagnetic resonance (EPR) oxygen imaging was investigated for its power to predict the success of tumor control depending on tumor oxygenation level and radiation therapy dose.

Methods and Materials—Thirty-four EPR oxygen images were obtained from the legs of C3H mice bearing 0.5 ml FSa fibrosarcomas under both normal (air breathing) and clamped tumor conditions. Under the same conditions as those during which the images were obtained, tumors were irradiated to a variety doses near the FSa TCD₅₀. Tumor tissue was distinguished from normal tissue using co-registration of the EPR oxygen images with spin-echo MRI images of the tumor and/or stereotactic localization. Tumor voxel statistics in the EPR oxygen image included mean and median pO₂, and the fraction of tumor voxels below the specified pO₂ values of 3, 6 and 10 torr. Bivariate logistic regression analysis using radiation dose and each of the EPR oxygen image statistics determined which best separated treatment failure from success.

Results and Conclusions—TCD₅₀ measurements were similar to those found in the literature for this syngeneic tumor. Bivariate analysis of 34 tumors demonstrated that tumor cure correlated with dose (p=0.004) and with <10 torr hypoxic fraction (p=0.023). Together, radiation dose and EPR image hypoxic fraction separate the population of FSa fibrosarcomas which are cured from those which fail, thus predicting curability.

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Keywords

EPR; EPR oxygen images; tumor oxygenation; tumor control; image guided therapy

Introduction

The oxygenation status of tissues appears to have profound effect on many aspects of the malignant state (1). Poor oxygenation – hypoxia -- selects mutant cells which survive the hostile environment, increasing the likelihood of the development of malignancy. Cells that survive a hypoxic environment have abnormal signaling and proliferation (2). Hypoxia stimulates angiogenesis and creates paths for cancer spread. Nearly a century ago hypoxic tissues were discovered to be resistant to radiation (3,4). Increased oxygen partial pressures or oxygen mimetic chemicals (5-7) overcome radiation resistance. One of the weaknesses of much of this clinical work is the assumption that most if not all of the tumors involved contained therapeutically relevant hypoxia (8,9). If well oxygenated tumors could be distinguished from those with significant hypoxia, the role of hypoxic modification would be substantially clarified. Moreover, an accurate quantitative oxygen image would allow therapy to be individualized on the basis of oxygenation status.

Strong correlation has been demonstrated between electrode measurements of low oxygen concentration and failure in radiation treatment of human head and neck cancer, cervical cancer and sarcoma (10-14). For example, in human cervical cancer, actuarial recurrence free survival at 70 months for median $pO_2 > 10$ torr is 69%; for median $pO_2 < 10$ torr, it is 37% (11). Clearly, diatomic oxygen levels demonstrate strong correlation with treatment outcome.

To date, imaging hypoxia in human cancers has been accomplished with positron emission tomography (PET) and with BOLD MRI. Radiotracer techniques involve reductive retention of tracer in hypoxic cells in tumors. ^{18}F -Misonidazole PET has shown promising correlation with treatment outcome in the head and neck cancer (15-17), although confounding results from lung cancer treatment exist (18). Rischin et al. (19) found a strong correlation between tumor uptake of ^{18}F -Misonidazole with PET and the success of curative therapy combining radiation with the hypoxic toxin tirapazamine. Chao et al. (20) demonstrated a technique to guide intensity modulated radiation therapy (IMRT) dose “hot spots” to Cu-ATSM PET hot spots to enhance radiation cure of tumors containing hypoxic regions. Therapeutic outcome relation with BOLD is not as easily found in the literature. Data indicate that BOLD signal not only depends on hemoglobin dissociation but also on vascular caliber and is preferentially sensitive to better perfused portions of tumors (21). In addition, BOLD MRI suffers from multiple confounding artifacts.

These oxygen imaging techniques lack a clear quantitative relationship between the image values and the dose increase necessary to overcome the effect of the hypoxia they show. EPR spectroscopic images, where quantitative oxygen concentration is obtained directly from the spectral linewidth of a suitable spin probe, do not suffer from this limitation. Non-imaging EPR spectroscopy using particulate materials has been shown to provide a highly sensitive quantitative measure of tissue oxygenation *in vivo* (22,23). Techniques for *in vivo* imaging of oxygen in the tissues and tumors of living animals have been developed over the past decade (24-26).

Unlike the reductive retention of tracer reported by PET or the non-quantitative images of BOLD MRI, EPR oxygen images are quantitative measures of tissue oxygenation. The quantitative nature of EPRI oxygen images means that they are repeatable, so successive oxygen images can be compared. Moreover, by providing a quantitative measure of local tissue

hypoxia, these images can be used to predict a specific dose increase that might convert an hypoxic tumor region from one that would fail radiation treatment to one that could be cured with radiation treatment. Given recent advances in radiation therapy delivery that provides controlled, heterogeneous tumor doses (20), such an image could guide dose painting based on the extent of hypoxia in various regions of the tumor. This has the potential to improve radiation therapy delivery, significantly increasing cure rate, reducing the toxicity of such treatment or both.

We have previously demonstrated good correspondence between oxygen measurements with an Oxylite™ fluorescence quenching measurement of oxygen tension and the values of the EPR oxygen image at the same point in a tumor (27). From a 4D oxygen EPR image, a large number of voxels can be measured and statistics can be obtained from a leg borne mouse tumor model. The abundance of tumor oxygenation data in each image stimulated the present series of measurements in mouse tumors to explore the role of both dose and fraction of hypoxic tumor voxels in an EPR oxygen image in predicting the cure of radiation therapy. The approach involved determining the dose at which 50% of tumors were cured (TCD₅₀). Following an oxygen image, mice were treated with doses above and below the TCD₅₀. Hypoxic fractions in tumors were increased using tourniquets to clamp the tumor blood supply for both image and treatment. A clear correlation was seen both between dose and cure and between hypoxic fraction and cure as independent variables in a bivariate analysis.

METHODS

Animals & Tumors

Thirty-four six to eight-week-old C3H/HeN:Hsd (Harlan Sprague-Dawley, Indianapolis, IN) female mice were injected intramuscularly with 5×10^5 FSa F9 generation fibrosarcoma cells (provided as F6 generation cells by Kathryn Mason, M.D. Anderson Hospital, Houston, TX) into the right hind leg. The tumors grew to 350–500 μ l in size in 7–10 days. Mice were imaged and treated with radiation under three different conditions: air breathing with no manipulation (n=12), air breathing with a soft clamp on the leg (n=12), and air breathing with a hard clamp on the leg (n=10). From gentle pressure on the limb, 2½ turns of a ¼–20 screw to tighten a fishline noose were used for a soft clamp and 3 turns were used for a hard clamp.

Tumor control

Tumors were measured twice per week. Local failure was declared when a tumor reached twice its original volume based on three crossed diameter measurements. Tumors were deemed to have been controlled after 90 days post radiation. Generally, tumors that were controlled completely regressed post radiation.

Radiation

Radiation doses were delivered in a single fraction given locally to the tumor, using 250 KVp X-ray from a Phillips RT 250 (Phillips, East Haven, CT) hardened with 0.5 mm Cu giving a HVL 1.9mm Cu. Dose rate was ~6 Gy/min. Fraction magnitudes between 21.1 Gy and 52 Gy were determined based on prior measurements of the TCD₅₀ for tumors from air breathing animals. Those measurements indicated a TCD₅₀ of $33.8 \pm 0.6/-0.2$ Gy for animals breathing air, with no oxygenation manipulation, consistent with values in the literature. (28) Tumors were immobilized for radiation with vinyl polysiloxane dental mold cast (GC America Inc, Alsip, IL), used also for EPR imaging. All other aspects of animal preparation for radiation were identical to that for imaging. For animals whose tumors were clamped, radiation was performed fifteen minutes after EPR imaging, giving the animals a five minute respite from tumor clamping before reclamping to make the image and radiation conditions as consistent as possible. For those animals treated with a tourniquet, a preliminary oxygen image was

obtained without the tourniquet, referred to as the air breathing control. Comparisons of day 0 and day 3 oxygen images were made between air breathing controls.

Oxymetric Spin Probe

For each image, 50 mg per 25 g mouse (2 g/kg equivalent to 1.4 mmol/kg animal weight) of OX063 trityl (methyl-tris[8-carboxy-2,2,6,6-tetrakis[(2-hydroxyethyl)]benzo[1,2-d:4,5-d']bis[1,3]dithiol-4-yl] trisodium salt, molecular weight = 1,427) (GE Healthcare, London, UK) was injected in 0.5 ml of deionized water. Conditions were: pH: 7.6, and tonicity 280 mOsm. The clamping of the tumor was delayed 5 minutes from spin probe injection to allow systemic distribution.

Anesthesia, Immobilization, and Preparation of the animals: Animals were prepared for the imaging as described in [27,30]. Briefly, animals inhaled sufficient isoflurane vapor (1–2%) to achieve normal breathing at 1 hertz and absence of voluntary movement. Vinyl polysiloxane dental mold material, encompassing one half of the circumference of the leg was used to immobilize the tumor bearing leg. The anesthesia started 40 minutes before EPR imaging. The skin temperature was maintained at 33.5°C using opposed heating lamps and measured with a Physitemp (Clifton, NJ) digital thermometer, during all procedures.

EPR Imaging

EPR oxygen images were obtained just prior to tumor radiation and three days after radiation where possible. Third day images were limited by difficulty in intravenous. canulation of the animal for the second image. 4D EPR images were obtained using a spectroscopic imager with an air-core magnet and Anderson type gradient coils (29) operating at 250 MHz and 9 mT as described previously.(30) The Zeeman field was overmodulated at 4.98 KHz with 17 μ T amplitude, enabled by an accurate line shape simulation to increase signal-to-noise ratio (SNR) without compromise in the determination of the intrinsic line width. (31,32) Other conditions include a loaded Q of 100–150 and nonsaturating power of 25 μ W.

EPR oxygen images were acquired and reconstructed using 4D tomography. Spectra were acquired with multiple fixed gradients, referred to as projections. 256 field points, each acquired in 3ms with a 3 ms lock-in time constant, were scaled to uniformly populate the projection field interval determined by each gradient (33). These were subjected to a 4-point Gaussian filter and then subsampled to 64 points. A field of view of $3\sqrt{2}$ cm was used, giving a voxel size of 0.66 mm (21,33). Projections were obtained with 14 gradient amplitudes, with multiple field sweeps to partially compensate for low SNR at high gradient amplitudes, and 66 directions evenly spaced in solid angle (34) requiring 48 minutes. Spectroscopic image reconstruction used filtered back-projection as described previously (21,27,33,34). Image voxels with spectral amplitude less than 15% of the maximum, showing areas of deficient vascular access, were discarded.

Oxygen concentration in each voxel was computed from the known, calibrated relationship between OX063 spectral linewidth and oxygen concentration (21). The oxygen maps have pO_2 resolution of ~ 3 torr and a spatial resolution of ~ 1 mm. (34) .

Location of Tumor Voxels in the EPR Image

a. Co-registration with MRI

Holes were molded in the vinyl polysiloxane (VPS) cast that tightly accommodated two or three 1 mm ID, 3–4 cm long borosilicate glass sample tubes containing either 10 mM OX063 (EPRI) or water (MRI) as fiducials for co-registration of EPRI with MRI as shown in Fig. 1. Rapid Acquisition with Refocused Echoes (RARE) spin-echo images, were acquired at 4.7 T

on an Omega Bruker/GE imager (Billerica, MA) with the following parameters: TR = 3000 ms, effective TE = 56 ms, FOV = 3.0 cm, matrix size = 256×256 , slice thickness = 1 mm, NEX = 1, rare factor = 8. The MRI with the water fiducials (shown as a 3D surface in Fig. 2a) was co-registered with an image of the EPRI fiducials represented as a wire mesh in Fig. 2a. Also indicated in Fig. 2a is the surface of the mouse leg from the MRI. Co-registration of EPRI and MRI images was done in several steps, using in-house software written in Matlab 7 (Mathworks, Cambridge, MA) allowing human intervention. Initial registration estimate was done based on alignment of surfaces of the external fiducials. Then a manual fine-matching of the tumor EPRI and MRI surfaces was performed, going through several slices within the tumor volume in three directions. A slice of the EPRI with MRI defined tumor voxels is shown as the red contour in Fig. 2b.

b. Stereotactic location of the tumor

Three perpendicular tumor dimensions were measured for each tumor. A needle was mounted in a stage fixed to the imager that could be precisely adjusted in three dimensions. The tip of the needle was moved to several precalibrated reference positions on the resonator whose positions were known in the image coordinates. The needle tip was then moved to 12–15 locations on the adjacent surface of the leg borne tumor (“tumor touch”). An ellipsoidal surface with major axes equal to the measured crossed tumor dimensions was fit to the surface locations provided by the stereotactic needle locator. Using the reference positions, the ellipsoid was transformed into the EPRI oxygen image coordinate system. The tumor voxels were defined as those enclosed by this ellipsoid with its dimension along the leg thickness direction decreased by 4 mm, determined by comparison with MRI. A typical comparison between the MRI determined tumor contour and the stereotactically determined tumor surface is shown in Fig. 2b.

Statistical Methods

Logistic regression was used to evaluate the effect of radiation dose and oxygen image-based characteristics on clinical outcome (treatment success or failure). Mathematically, the logistic regression model can be written as

$$\ln(p/(1-p)) = \beta_0 + \beta_1 * \text{Dose}_i + \beta_2 * \text{Oxygenation}_i + e$$

where p is the probability or odds of cure, β_1 and β_2 are the regression coefficients for dose and hypoxic fraction, and e is the error term. Odds ratios (OR's) for the fitted models are reported. A likelihood ratio test was used to assess whether the addition of the oxygenation variable to the model improved the model fit relative to the model with dose as the only predictor. The McFadden's pseudo- R^2 , a logistic regression goodness-of-fit measure analogous to R^2 in ordinary least squares regression, is reported to reflect the magnitude of the improvement (35). Several tumor oxygenation characteristics were considered, including mean and median oxygen values of tumor voxels, and fraction of tumor voxels with less than 3, 6 and 10 torr oxygen tension (HF3, HF6, and HF10). Separate bivariate logistic regression models were used to determine which of the tumor oxygenation characteristics is more strongly associated with outcome, adjusting for dose. The best-fitting model was selected using the Bayesian Information Criterion (BIC).

Based on the logistic regression model, predicted outcome was classified as “success” if the predicted probability of success was greater than 0.5, and classified as “failure” otherwise. The misclassification rate was estimated as the proportion of observations where the observed and predicted outcome differed.

Estimate of the dose-hypoxic fraction line which partitions failure from success was obtained by setting the predicted probability of success and failure equal $p=0.5$, resulting in $\ln(p/(1-p))$

=0. Based on the fitted logistic regression model, the equation for this partition line can be written as

$$Oxygenation = -(\widehat{\beta}_0 + \widehat{\beta}_1^* \text{Dose}) / \widehat{\beta}_2,$$

where $\widehat{\beta}_0$, $\widehat{\beta}_1$ and $\widehat{\beta}_2$ are the estimated regression coefficients from the logistic regression model.

P-values <0.05 were considered significant. Analyses were performed using the STATA 9 statistical package (StataCorp, LP, College Station, TX).

Results

Spin probe distribution

Distribution of OX63 through the animal occurs in 3–5 minutes (36). We found that the maximum concentration in the tumor occurs at this time. The clamping of the tumor was delayed 5 minutes from spin probe injection to allow systemic circulation. Analysis of the distribution of voxels where the intensity failed to achieve 15% of the maximum intensity showed the numbers to be lower in the clamped tumors but not significantly lower. No functional difference between the legs of clamped and unclamped mice was observed other than some transient swelling.

Example of an EPR oxygen image

EPR oxygen images present a wealth of information, typically 6000 voxels from the mouse leg of which 1500 are located in the tumor. A surface rendering from the intensity of the signal from a typical EPR oxygen image is shown in Fig 3. Two planes showing the oxygenation of the tissue are also shown with a quantitative color bar. This is a representation of the type of data from which the results and conclusions of this paper are derived. Typical large oxygen tension gradients are seen over relatively few voxels as shown in Fig. 3.

Distinguishing tumor voxels

As described in the Methods section, two methods were used to identify tumor voxels: mapping MRI tumor volumes into EPRI via image registration, and stereotactic tumor touch. Comparison of tumor contours in a single slice from a typical EPR amplitude image where both methods were used is shown in Fig. 2b. Agreement was generally good, typically a pixel or two different, which corresponds to 0.66 to 1.33 mm positional disagreement. Ten of the thirty-four images were obtained using MRI and stereotactic “tumor touch” together. Twenty-one EPRI used stereotactic “tumor touch” alone. Three of the EPRI oxygen images used MRI co-registration alone. As shown in Table 1 there was virtually no effect on the statistical significance of the results when the two different definitions of the tumor voxels were used.

HF10 plus radiation predicts tumor cure

A total of 18 mice out of 34 showed tumor control beyond ninety days post treatment, nearly all shrinking to non-palpable at ninety days. For statistical analysis oxygen information was taken only from tumor voxels, selected from the leg using co-registered MRI where available (n=13) or from the stereotactic “tumor touch” technique (n=21). Bivariate logistic regression models of tumor control vs. each statistic from the tumor voxels of the 34 oxygen images along with the dose given was performed for the following oxygen image statistics: HF3, HF6, HF10, mean tumor oxygen tension, or median oxygen tension. Results for each of these pO₂ statistics are shown in Table 1. Using bivariate logistic regression models with each of the oxygenation measures, the model with dose and HF10 had the best fit. Dose was positively associated with tumor control (OR=1.64, p=0.004), and HF10 was negatively associated with tumor control

(OR=0.80, $p=0.023$). Fig. 4 shows a scatter plot of both the controlled tumors and the failed tumors vs HF10 and radiation dose. The equation of the classification line shown in Fig. 4 is

$$\text{HF10 (in \%)} = 63.96\% - 2.18\%/\text{Gy} \times \text{dose}$$

or 0.46 Gy per percent increase in HF10. The minimum cure dose is approximately 29.4 Gy (corresponding to 0% HF10). The misclassification rate for this model was 8.8% (3 in 34).

The improvement resulting from the addition of measurement of HF10 in the EPR oxygen image is indicated by the pseudo- R^2 which increased from 0.38 for the model with dose as the only predictor, to pseudo- $R^2 = 0.59$ when HF10 was added. The likelihood ratio test confirmed that the bivariate model provides better fit ($p=0.002$).

We tested the potential importance of using the stereotactic “tumor touch” technique alone for distinguishing tumor voxels from normal tissue voxels. The estimated odds ratios in the bivariate logistic regression model of dose and hypoxic fraction using only “tumor touch” location was OR=0.83, virtually identical to the estimated OR using both “tumor touch” and MRI tumor measurements.

Day 3 images generally show increased tumor oxygenation

Air breathing oxygen images were also obtained 3 days after radiation treatment for 17 animals. These were compared with normal air breathing animals or the air breathing controls for day 0 animals treated with a tourniquet. The principal reason for the diminished number of images was difficulty with intravenous cannulation and consequent inability to inject the trityl spin probe for the second image. The change in oxygenation of these tumors is illustrated in Fig. 5. Arrows have been added to indicate the direction and magnitude of oxygen change on day 3. As can be seen, the majority of tumors had a decreased HF10, i.e. an increase in oxygenation. The average decrease in the HF10 was 4.5% which was statistically significant ($p=0.014$, based on paired t-test). Also clear is the fact that the magnitudes of the HF10 changes were much larger in the direction of increased oxygenation on day 3, compared to the decreases.

The significance of both dose (OR=1.2, $p=0.06$) and HF10 (OR=0.74, $p=0.176$) correlation with cure was reduced for these day 3 images, although the magnitude of the effect was similar to the day 0 model. The misclassification rate for the day 3 model increased to 17.6% (3/17).

HF10 shows the strongest correlation with cure

Table 1 shows the odds ratios, statistical significance and overall model fit summaries obtained from bivariate logistic regression models of tumor control vs. dose and various pO_2 parameters obtained from the EPRI tumor oxygen images. The model with the lowest BIC was selected, and the corresponding pseudo- R^2 and missclassification rates are provided in Table 1. A range of levels of pO_2 threshold were used to define the hypoxic fraction. The use of HF10 resulted in the best-fitting model (pseudo- $R^2=0.59$, misclassification rate 8.8%). Models with pO_2 threshold of 6 torr and 3 torr provided somewhat worse overall fit. Mean and median pO_2 in the tumor showed a much weaker association with tumor control, with substantially lower pseudo- R^2 and increased misclassification rates. However, given the large gradients and pO_2 variations in the image, as in Fig. 3, such overall descriptors of the oxygenation might not be expected to show good correlation with tumor cure.

Discussion

HF10 provides the most significant pO_2 statistic to distinguish cured from failed tumors

As can be seen from Figure 3, EPRI provide a comprehensive survey of the pO_2 within a tumor. The spatial resolution of the image is 1 mm and the oxygen resolution is 3 torr. Fig. 3 shows

large gradients in the oxygen concentration. To allay concerns that this was due to image artifact, we previously compared point by point oxygen tensions measured using an OxyLite oxymeter with those measurements at the same points made with the EPR oxygen image. These correlated well, indicating that high oxygen gradients are characteristic of FSa tumors (27). Our results show that it is important to have a comprehensive survey of the oxygen tensions. The large number of tumor measurements can provide a number of different statistical summaries of the data, which can be used to search for best correlation with treatment outcome. It allows us to conclude that HF10 is the best statistical descriptor of the tumor hypoxia.

Partitioning of tumors using dose and HF10 is remarkably clear

The data from the EPR oxygenation images with radiation dose provide strong evidence for dependence of FSa mouse fibrosarcoma curability on image hypoxic fraction and single doses of radiation therapy near the TCD₅₀. As shown in Fig. 4 and corroborated by the logistic regression analysis there is nearly complete partitioning of tumors which are controlled from those which fail the therapy on the basis of the HF10 together with the radiation dose (8.8% misclassification rate). Logistic regression also shows that a pair of values for each tumor: HF10 and radiation dose given is a strong determinant of the tumor failure after radiation therapy, even with a relatively small population, 34 animals.

The day 3 post radiation oxygen images show variations in reoxygenation

The reduction in the significance of the separation between controlled and failed tumors is partially due to the reduced number of images. However, as shown in Fig. 5 reoxygenation reduces the significance of the HF10 with dose as a tumor cure discriminant. But it is also clear that the change in hypoxic fraction is not uniform either in direction or magnitude. Of the 17 changes, four show increased hypoxia and twelve show decreased hypoxia. Eight of the 17 changes were less than 5 torr, three with decreased oxygenation. Defining small changes as no reduction in hypoxia, 9 tumors of the 17 tumors imaged on day 3 failed to reoxygenate. Reoxygenation is thus not assured. EPR images can provide an important means by which to measure whether reoxygenation is taking place. Reoxygenation blurs the pre-treatment distinction provided by the EPR oxygen image HF10. But the image provides a means by which to select those tumors which undergo reoxygenation, to quantify the extent of that reoxygenation, and to locate that reoxygenation in the tumor. This may lead to the development of more potent schemes for adapting subsequent fractions of therapy to the actual response of an individual tumor or tumor portion.

EPR oxygen images may provide a means to determine hypoxic regions in human tumors to guide IMRT therapy

The data here refer to the control of whole tumors subjected to single doses of radiation. One can regard larger tumors as consisting of multiple 0.5 ml subvolumes, the volumes of the tumors in this study, with differing HF10. Given evidence of a large HF10, that portion of the tumor could be given an extra dose of radiation to bring its total dose to a position to the right of the boundary line in Fig. 4, "dose painting" (37). Other hypoxia image assays dependent for example on reductive retention of radiotracer (15-17), are not quantitative and cannot provide such specific dose enhancements that could be applied to regions that can be defined with EPRI to have specific hypoxic fractions. This quantitative hypoxia assessment can either guide the application of additional dose to enhance the overall cure probability for a given treatment or allow a smaller basic dose to be given to the tumor and surrounding normal tissues for a given level of cure, reducing side effects from therapy. In either case, the therapeutic ratio is enhanced.

Because EPR oxygen images are quantitative, values from images obtained at different times may be compared. Such repeated images might be used in fractionated radiation therapy to

direct increased doses to regions that do not appear to reoxygenate within a given tumor. If EPR oxygen imaging can be scaled to human tumor measurements, these data suggest an important clinical application of the technique in such an adaptive image guidance scheme.

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Figure 1.
Tumor-bearing leg in vinyl polysiloxane cast in 16 mm resonator with 2 fiducials.

Figure 2a.

Co-registration of EPRI fiducial images (red wire mesh) and MRI fiducial images (green shaded surface). Also shown in green is the whole leg surface from MRI.

Figure 2b.

Comparison of tumor contours from MRI (red contour) and the stereotactic needle ("tumor touch", yellow contour) in an EPRI image slice. Pixel volume is $(0.66 \text{ mm})^3$.

Figure 3. Surface rendering of the intensity from an EPR image is shown in tan. Tissue pO_2 values are represented as colors in the orthogonal planes. A quantitative color bar relates color to pO_2 .

Figure 4. Scatter plot of controlled tumors (o) and tumors that failed radiation (x): hypoxic fraction -- % EPR oxygen image voxels less than 10 torr (HF10)– prior to radiation vs Dose (Gy). S indicates soft tourniquet clamped tumors, H – hard tourniquet, U – unclamped tumors.

Figure 5. Scatter plot of 17 tumors imaged both on day 0 and day 3. HF10 -- % EPR oxygen image voxels less than 10 torr – prior to radiation of controlled tumors (o) and tumors that failed radiation (x) vs Dose (Gy), with arrows indicating the direction and magnitude of HF10 change on day 3 relative to day 0. S indicates soft tourniquet clamped tumors, H – hard tourniquet, U – unclamped tumors.

Table 1

Results of bivariate logistic regression of the effect of radiation dose and various tumor oxygenation statistics on tumor control. The oxygenation statistics were obtained from the EPR oxygen image acquired prior to treatment. P-value less than 0.05 suggest significant correlation with tumor cure. "Tumor touch" refers to the stereotactic registration described in the Methods section. N=34 EPR images were used.

Oxygenation statistic derived from the EPR image	Odds Ratio (p-value) of oxygenation statistic	p-value of radiation dose	Misclassification Rate (%)	pseudo-R ²
HF10	0.80 (0.023)	1.64 (0.004)	8.8	0.59
HF10 "tumor touch" only (n=31)	0.80 (0.025)	1.60 (0.006)	9.7	0.56
HF6	0.76 (0.039)	1.57 (0.005)	11.8	0.57
HF3	0.73 (0.054)	1.58 (0.005)	14.7	0.57
Mean pO ₂	1.09 (0.177)	1.41 (0.003)	14.7	0.43
Median pO ₂	1.12 (0.135)	1.46 (0.004)	14.7	0.45