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## Repeated tumor pO<sub>2</sub> measurements by multi-site EPR oximetry as a prognostic marker for enhanced therapeutic efficacy of fractionated radiotherapy

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### Abstract

**Purpose**—To investigate the temporal effects of single or fractionated radiotherapy on subcutaneous RIF-1 tumor pO<sub>2</sub> and determine the therapeutic outcomes when the timing of fractionations is guided by tumor pO<sub>2</sub>.

**Methods**—The time course of the tumor pO<sub>2</sub> changes was followed by multi-site electron paramagnetic resonance (EPR) oximetry. The tumors were treated with a single 10 Gy, 20 Gy, and 10 Gy × 2 doses and the tumor pO<sub>2</sub> was measured repeatedly for six consecutive days. In the 10 Gy × 2 group, the second dose of 10 Gy was delivered at a time when the tumors were either relatively oxygenated or hypoxic. The changes in tumor volumes were followed for nine days to determine the therapeutic outcomes.

**Results**—A significant increase in tumor pO<sub>2</sub> was observed at 24 hr post 10 Gy, while 20 Gy resulted in a significant increase in tumor pO<sub>2</sub> at 72 to 120 hr post irradiation. The tumors irradiated with a second dose of 10 Gy at 24 hr, when the tumors were oxygenated, had a significant increase in tumor doubling times (DT), as compared to tumors treated at 48 hr when they were hypoxic (p<0.01).

**Conclusion**—Results indicate that the time of tumor oxygenation depends on the irradiation doses, and radiotherapeutic efficacy could be optimized if irradiations are scheduled at times of increased tumor oxygenation. *In vivo* multi-site EPR oximetry could be potentially used to monitor tumor pO<sub>2</sub> repeatedly during fractionated schemes to optimize radiotherapeutic outcome. This technique could also be used to identify responsive and non-responsive tumors, which will facilitate the design of other therapeutic approaches for non-responsive tumors at an early time points during the course of therapy.

### Keywords

EPR oximetry; pO<sub>2</sub>; Oxygenation; Radiotherapy; RIF-1

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## INTRODUCTION

The level of oxygen in tumors has a profound effect on radiotherapeutic efficacy, with a decrease in responsiveness of up to a factor of three when the tumor  $pO_2$  (partial pressure of oxygen) decreases from radiobiologically oxidic levels (greater than 15–20 mm Hg) to profound hypoxia (1). Tumor  $pO_2$  also can change during the course of fractionated radiotherapy. In experimental tumors, single small radiation doses often lead to increased oxygenation, whereas decreased oxygenation has been observed at higher doses (2–9)

Given the fact that changes in tumor oxygenation occur during fractionated radiotherapy, an appropriate choice of the intervals between fractions based on tumor oxygen status should enhance therapeutic outcomes. However, this requires an appropriate technique for repeated  $pO_2$  measurements to determine the time-course of tissue  $pO_2$  changes of the tumors undergoing therapy. This is vital because tumor oxygenation levels can be affected by several factors such as tumor growth, radiation, and changes in tumor vasculature, which will result in a very unpredictable variation in tumor  $pO_2$  over time.

Tumor re-oxygenation after single-dose or during fractionated radiotherapy has been investigated previously using assays for hypoxic fractions, but little is known about the time-course of the actual changes in tumor oxygen during treatment. Using the comet assay, Oliver *et al.* reported a decrease in the hypoxic fraction of murine SCC VII tumors from 10% in untreated animals to 6% at 6 hr after a single dose of 10 Gy (10). An increase in the hypoxic fraction (6%–80%) of SCC VII tumors immediately after a single dose of 10 Gy was observed, and this decreased to approximately 2% at 6 hr post irradiation (11). Some very useful information has been obtained in human tumors by using oxygen electrodes (12,13), but repeated measurements have not been possible so far due to lack of techniques that can provide repeated  $pO_2$  measurements. The oxygen electrodes involve a significant degree of invasiveness, have limited sensitivity, and most importantly, cannot be used for repeated measurements (14). Repeated measure of tumor  $pO_2$  during fractionated radiotherapy will provide vital  $pO_2$  information that could be exploited to optimize therapy by scheduling subsequent doses at times of optimal tumor  $pO_2$ .

EPR oximetry is a relatively new technique that has been used extensively for  $pO_2$  measurements in animal models (2,15,16) and is now being developed for clinical use (17). One particular advantage of EPR oximetry is its ability to provide detailed  $pO_2$  information repeatedly from the same tumor, which makes it ideal for measurements over a course of radiation treatment (18,19). The recent development of multi-site EPR oximetry has further expanded its *in vivo* application by allowing simultaneous  $pO_2$  measurements at multiple sites in a tissue of interest (15,16). We have investigated the temporal effects of single or fractionated radiotherapy on tumor oxygenation of subcutaneous RIF-1 tumors using repeated multi-site *in vivo* EPR oximetry. This information could potentially be used to optimize therapeutic outcome and to also identify responding and/or non-responding tumors. We have tested this hypothesis by determining the radiotherapeutic outcome when the timing of fractionations is guided by tumor  $pO_2$ . The prognostic capability of EPR oximetry is investigated by identifying responding and non-responding tumors based on their tumor  $pO_2$  and their radiotherapeutic outcome. The results indicate a significant increase in tumor  $pO_2$  post irradiation and a significant increase in doubling time when the tumors are irradiated at times of increased tumor  $pO_2$ .

## MATERIALS AND METHODS

### Animals and Tumor Models

All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Dartmouth Medical School. The radiation-induced fibrosarcoma tumor (RIF-1) cells were a gift from Dr. J. B. Mitchell's laboratory at the National Cancer Institute. The cells were cultured *in vitro* in RPMI 1640 media supplemented with 10% FBS, glutamine, and antibiotics. The procedure for tumor inoculation has been described previously (15,16). Briefly, the subcutaneous RIF-1 tumors with an average diameter of 6 – 8 mm were obtained by injecting 50  $\mu$ l of  $5 \times 10^5$  RIF-1 cells in the left posterior flank of female C3H mice (18–20g, Charles River Lab, Wilmington, MA).

### Paramagnetic Probe

LiPc (lithium phthalocyanine) crystals were synthesized in our laboratory; the physico-chemical properties of LiPc crystals have been described previously (20). The LiPc crystals have a single sharp EPR line whose width is highly sensitive to  $pO_2$ . The EPR spectra reflect the average partial pressure of oxygen on the surface of the crystals and allow measurements of  $pO_2$  in the tumor tissue using one or more crystals with a total diameter of  $\sim 200 \mu$ m. The procedure for LiPc injection has been described in earlier publications (15,16). Briefly, the mice were anesthetized using 1.5% isoflurane with 30%  $FiO_2$  (fraction of inspired oxygen), and two aggregates of the LiPc crystals (30–50  $\mu$ g each) were implanted at a depth of 2 mm and at a distance of 4 mm into each tumor using 25-gauge needles. One day after the LiPc implantation, multi-site EPR oximetry was used to measure tissue  $pO_2$  of the subcutaneous tumors (baseline  $pO_2$ ).

### Electron Paramagnetic Resonance (EPR) Oximetry

The EPR oximetry was performed on an L-band (1.2 GHz) EPR spectrometer utilizing a microwave bridge and an external loop resonator specially designed for *in vivo* experiments (21). A set of coils capable of generating a magnetic field gradient in the Z- direction with a magnitude up to 3.0 G/cm was used to separate the EPR spectra of the two implants in each tumor (15,16,22). The spectrometer parameters were: incident microwave power, 2 mW; magnetic field center, 425 gauss; scan range, 2 gauss; modulation frequency, 24 kHz; modulation amplitude was one-third of the EPR line width with scan time of 10 seconds. We averaged 5 – 6 scans to enhance the signal to noise ratio. The EPR line widths were converted to  $pO_2$  using a calibration curve determined for the LiPc crystals used in this study (15,16, 20).

### Experiment Protocol

Mice were assigned randomly into 5 groups: (A) Control (sham-irradiated tumor on day 0, n = 9), (B) tumors irradiated with a single dose of 10 Gy (10 Gy on day 0, n = 16), (C) single dose of 20 Gy (20 Gy on day 0, n = 9). Based on the changes in tumor  $pO_2$  observed in these groups, we designed two more groups: (D) 10 Gy  $\times$  2 oxygenated (10 Gy on day 0 and 10 Gy at 24 hr post first dose, n = 24), and (E) 10 Gy  $\times$  2 hypoxic (10 Gy on day 0 and 10 Gy at 48 hr post first dose, n=10). n is the total number of LiPc implants in each group i.e. total number of  $pO_2$  measurements in each group at each time point.

For tumor  $pO_2$  measurements, the mice were anesthetized (isoflurane 1.5%, 30%  $FiO_2$ ) and positioned in the EPR magnet. The rectal temperature of the animal was maintained at 37  $^{\circ}$ C by a heated water pad and warm air blower. EPR oximetry measurements were carried out for 30 minutes in each mouse to determine the baseline tumor  $pO_2$ . The animals were then moved to the irradiator bed [Varian Linear Accelerator (Clinac 2100C); Energy: 6 Mev; Applicator:

6 cm×6 cm], and the beam was focused on the tumor. Appropriate lead shields were used to prevent irradiation to the normal tissue of the mice. The tumor pO<sub>2</sub> measurements post irradiations were repeated at 4 hr, 24 hr, 48 hr, 96 hr and 120 hr post irradiation. The animals of groups D and E received a second dose of 10 Gy after 24 hr and 48 hr respectively. The tumor volume was measured by a standard procedure ( $\text{volume} = \pi/6 \times \text{length} \times \text{width}^2$ ) for 9 consecutive days. Tumor doubling time (DT) is determined by using a statistical modeling approach described below. Multi-site EPR oximetry procedure requires a minimal distance of 4–5 mm between implants, so the initial diameter of the tumor was 6–10 mm in these studies. After 5 days of experiments, the tumor size reached the maximum tumor load limit per individual mouse as per the guidelines of IACUC at Dartmouth (should not be more than 10% of total body weight). This limited our experimental procedures for carrying out growth delay measurements therefore we used the modeling approach to determine DT.

**Statistical Analysis**—A paired t-test was used to determine statistical significance of changes in pO<sub>2</sub> and tumor volume within the group and an unpaired t-test was used to determine the statistical significance between groups. The paired comparison reduces the animal to animal heterogeneity and eliminates differences of the baseline pO<sub>2</sub>. The tumor growth delay was modeled using an exponential mixed model (23) on the log scale, which was estimated by function  $l_{me}$  in the statistical package S-Plus 6.1 (Insightful Inc., Seattle, WA). Assuming an exponential growth, DT was computed as  $DT = \ln 2/a$ , where “ $a$ ” is the rate of tumor volume growth obtained from  $l_{me}$ . The standard error for DT was estimated using the delta-method as described by Rice (24). The tests were two-sided, and a change with a p value < 0.05 was considered statistically significant. This approach has been used previously to estimate DT (25–27). All data are expressed as Mean ± SE and n is the total number of LiPc implants (i.e. pO<sub>2</sub> measurements at each time point) in each group and N is the total number of animals in each group.

## RESULTS

### Measurements of the time course of tumor oxygenation before and after single radiation doses

First we investigated the effect of single doses of 10 Gy and 20 Gy on RIF-1 tumor pO<sub>2</sub> and tumor growth. Figure 1 summarizes the average tumor pO<sub>2</sub> during six days of repeated measurements. There was no significant difference in the baseline pO<sub>2</sub> between the two LiPc deposits of each tumor; therefore, the pO<sub>2</sub> reported by the two LiPc deposits were pooled in each group. No significant difference was observed between the mean baseline tumor pO<sub>2</sub> of the control (6.9 ± 1.0 mm Hg) and the treatment groups; 6.6 ± 0.7 mm Hg in 10 Gy group, and 6.8 ± 0.8 mm Hg in 20 Gy group. A significant increase in tumor pO<sub>2</sub> was observed at 24 hr post 10 Gy ( $p < 0.01$ ) Figure 1b, while no such change in tumor pO<sub>2</sub> was observed at this time point in tumors treated with 20 Gy (Figure 1c). However, a significant increase in tumor pO<sub>2</sub> was observed at 72 to 120 hr post 20 Gy irradiation ( $p < 0.05$ ).

### Measurements of the time course of tumor pO<sub>2</sub> before and after split dose radiation

Based on the results described above, the tumors were irradiated with 10 Gy on day 0 and at 24 hr when a significant increase in tumor pO<sub>2</sub> after first 10 Gy was observed (group D). Another group was irradiated with 10 Gy on day 0 and at 48 hr when the tumor pO<sub>2</sub> returned to baseline (group E). A significant increase in tumor pO<sub>2</sub> was observed at 4 hr ( $p < 0.05$ ) and from 24 hr to 120 hr ( $p < 0.01$ ) time points in group D, Figure 2b. A significant increase in tumor pO<sub>2</sub> at 24 hr ( $p < 0.05$ ) and at the 96 hr and 120 hr time points ( $p < 0.05$  and  $p < 0.01$ ) was observed in group E, Figure 2c.

### Effect of single or fractionated doses on tumor growth

To test our hypothesis that tumor pO<sub>2</sub> could be used as a prognostic marker to optimize fractionated radiotherapy by scheduling doses at times of increased tumor oxygenation, we investigated the growth inhibition of a control group (group A), tumors irradiated with a single dose of 10 Gy (group B), with 20 Gy (group C) or 10 Gy × 2 separated by a delay of 24 hr, at a time when oxygen is expected to be relatively higher (group D) or 10 Gy × 2 Gy separated by 48 hr, at a time when oxygen is expected to be relatively lower (group E). Table 1 shows the relative changes of RIF-1 tumor volume before and after irradiation of these groups. No significant differences in the initial tumor volume (prior to irradiation) of the control and treatment groups were observed. There was a significant decrease in the tumor volume from the baseline of the treatment groups from day 2 to day 8 ( $p < 0.01$ ) as compared to the control group. There also were significant differences between group D and group E on day 8 ( $p < 0.05$ ). The mean DT was  $2.9 \pm 0.1$  days for the control group,  $3.8 \pm 0.1$  and  $10.1 \pm 1.0$  days for groups B and C, respectively, Figure 3. The DT further increased significantly in the group irradiated with 10 Gy at day 0 and 24 hr time points (group D) to  $16.8 \pm 1.8$  days ( $p < 0.01$  compared with group E). On the other hand, the DT of the tumors irradiated with 10 Gy on 0 and 48 hr time points was only  $8.6 \pm 0.6$  days (group E).

We further investigated if the tumor pO<sub>2</sub> observed post first 10 Gy dose could be used as a prognostic indicator to predict therapeutic response during fractionated radiotherapy. The tumors of group D (10 Gy +10 Gy at day 0 and 24 hr) were divided into two sub-groups, based on their tumor pO<sub>2</sub> at 24 hr; D1, tumors with increased pO<sub>2</sub> <50 % of baseline pO<sub>2</sub>, (less responsive group) and D2 tumors with increased pO<sub>2</sub> ≥ 50 % of baseline pO<sub>2</sub> (more responsive group), and the changes in tumor volume in these two sub groups were compared. A significant difference in tumor pO<sub>2</sub> on day 1 ( $p < 0.05$ ) and in changes from the baseline tumor volume on day 8 ( $p < 0.05$ ) was observed between these two groups, Figure 4. These results indicate that within a group of tumors that received the same treatment, the tumor response (i.e. therapeutic outcome) to the subsequent dose could be predicted by the response of the tumor pO<sub>2</sub> to the first dose.

### Morphologic examination of the location of LiPc in the tumor

At the end of the experiment, gross and microscopic examination (H & E staining) of the tissue around the LiPc deposits confirmed that the deposits were in the interstitial compartment of the tumor tissue with no evidence of edema or infiltration of inflammatory cells (data not shown). Some accumulation of red blood cells and some necrotic cells around the LiPc deposits were observed in some samples, but these were not caused by the LiPc implants; rather, it reflects the normal histological pattern of the tumor. This is consistent with the previous report on the histological appearance of LiPc implants in RIF-1 tumors (28).

## DISCUSSION

The data reported here are the continuation of our systematic studies to characterize the changes in tumor pO<sub>2</sub> following irradiations. We have previously reported the tumor pO<sub>2</sub> changes following the administration of a synthetic allosteric modifier of hemoglobin (efaproxiral), and during carbogen or hyperbaric oxygen therapy, with the goal to use these interventions to enhance tumor oxygenation to improve radiotherapeutic efficacy (2,15,16,29). With the recent development of multi-site EPR oximetry, it is now possible to do simultaneous pO<sub>2</sub> measurements at multiple sites in a tissue (15,16). Given the heterogeneity in a tumor, the multi-site EPR oximetry approach is likely to provide a better assessment of average tumor pO<sub>2</sub> and its dynamics during various therapies. These results provide detailed information on the changes in tumor pO<sub>2</sub> during single and multiple irradiations by repeated pO<sub>2</sub> measurements using EPR oximetry for five consecutive days. The knowledge of the changes

in tumor pO<sub>2</sub> during radiotherapy is crucial for the optimization of fractionated radiotherapy as demonstrated here.

The baseline tumor pO<sub>2</sub> observed in these experiments is consistent with our previous reports (15,16). A statistically significant increase in tumor pO<sub>2</sub> at 24hr post 10 Gy was reproducible and consistently observed in all the irradiated groups, Figures 1 and 2. These results are in agreement with Znati et al. who reported an increase in tumor oxygenation when total doses of more than 10 Gy were used (3). Fuji et al. observed an increase in SCC VII murine tumors pO<sub>2</sub> at 6 hr post 10, 15 or 20 Gy irradiation, and the pO<sub>2</sub> continued to increase till 24 hr followed by a gradual decline (4). Using 2 fractions of 10 Gy each, O'Hara et al. assessed tumor pO<sub>2</sub> from a single ink implant (20 µl volume) and observed an initial decrease followed by an increase (re-oxygenation) of tumor pO<sub>2</sub> to the baseline level (pre-irradiation pO<sub>2</sub>) (2). No significant difference in DT was observed by O'Hara et al. in tumors with initial volume of 230 mm<sup>3</sup>. Sonveaux et al. reported that low doses of ionizing irradiation (6 Gy) induced a marked increase in mouse hepatocarcinoma (TLT) tumor blood flow and oxygenation (30). Results indicated a nitric oxide mediated effects of irradiation on the tumor vasculature and the irradiation of endothelial cells in a tumor is a key determinant of the effectiveness of radiotherapy. Crockart et al. used EPR and Oxilyte to investigate tumor oxygenation after a 2 Gy fraction in fibrosarcoma type II (FSaII) tumor models (31). An increase in pO<sub>2</sub> as early as 3 – 4 hr post irradiation due to decrease in oxygen consumption and an increase in oxygen supply was observed. The increase in oxygen delivery was found to be due to a decrease in interstitial fluid pressure (IFP) and radiation-induced acute inflammation. Several possible mechanism have been suggested for re-oxygenation such as reduced oxygen consumption by radiation-damaged cells (32), cell loss leading to tumor shrinkage (33), migration of hypoxic cells to oxygenated state (34), and improved microcirculation (35). Clinical studies of advanced carcinoma of the cervix have shown a better local control by radiotherapy in patients with tumor pO<sub>2</sub> greater than 10 mm Hg, as compared to patients with tumor pO<sub>2</sub> less than 10 mm Hg (36). Patients receiving radiotherapy for soft-tissue sarcoma showed a 70% frequency of distant metastases in tumors with pO<sub>2</sub> less than 10 mm Hg versus 35% in those with tumor pO<sub>2</sub> greater than 10 mm Hg (37). These findings indicate that the magnitude of the observed increase of oxygenation in the tumors has significant implications for enhancing the effectiveness of fractionated radiotherapy. This also appears to affect the probability of distant metastases.

The observed increase in tumor pO<sub>2</sub> on day 4, and day 5 after single 20 Gy or split 10 Gy × 2 is consistent with others reports (2,5–7). Goda et al. observed a decrease in tumor pO<sub>2</sub> initially (maximum decrease at 24 hr) followed by a gradual increase reaching a peak value at 72 hr after a single 20 Gy or split 10 Gy doses (8). An increase in tumor pO<sub>2</sub> as late as day 3 in NFSa fibrosarcomas treated with 25.5 Gy x-ray irradiation was observed by Fukawa et al. (9). However, only a few reports describe tumor oxygenation status during fractionated radiotherapy and how this information could be used to optimize fractionated radiotherapy.

Our results indicate a significant difference in DT of 10 Gy, 10 Gy × 2 and 20 Gy groups as compared to controls (Figure 3, Table 1). A significant increase in tumor pO<sub>2</sub> after 10 Gy and a significant increase in therapeutic efficacy was observed when the second dose of 10 Gy was delivered at time of increased tumor pO<sub>2</sub> (24 hr) as compared to the tumors irradiated at 48 hr time point (hypoxic). Furthermore, in 10 Gy × 2 group (D), the tumors with an increase in pO<sub>2</sub> of more then 50% from the baseline pO<sub>2</sub> had a significant increase in DT as compared to the group with pO<sub>2</sub> < 50 % of the baseline pO<sub>2</sub>. These results indicate that the changes in tumor pO<sub>2</sub> could be used as a prognostic indicator of tumor response to subsequent doses during the course of radiotherapy. This type of repeated pO<sub>2</sub> measurements might have a significant clinical application in identifying responsive and non-responsive tumors, and this vital information could than be used for an early intervention using other approaches for non-

responsive tumors. We did not see any correlation between the changes in tumor pO<sub>2</sub> and tumor volume over days in sham control group (data not shown). This is in agreement with observations in transplanted rat tumors (35), and our previous studies with the RIF-1 tumors in mice (15,16).

In conclusion, our results indicate a significant increase in tumor doubling time when fractionated radiations were scheduled at times of increased tumor oxygenation. The changes in tumor pO<sub>2</sub> post first dose during a fractionated regimen could also be used to identify less and more responsive tumors that received the same treatment. These results highlight the potential value of tumor pO<sub>2</sub> assessment during fractionated radiotherapy and provide evidence that tumor pO<sub>2</sub> could be potentially used to optimize and predict the therapeutic response. *In vivo* multi-site EPR oximetry was sufficiently sensitive to monitor the effects of single or fractionated radiation on tumor oxygenation. These results illustrate a unique and useful capability of *in vivo* multi-site EPR oximetry: obtaining repetitive and non-invasive measurements of tumor oxygenation from the same tumors during the course of therapy. This technique is currently being used to assess tissue pO<sub>2</sub> of superficial tumors in patients undergoing radiotherapy and/or chemotherapy (29).

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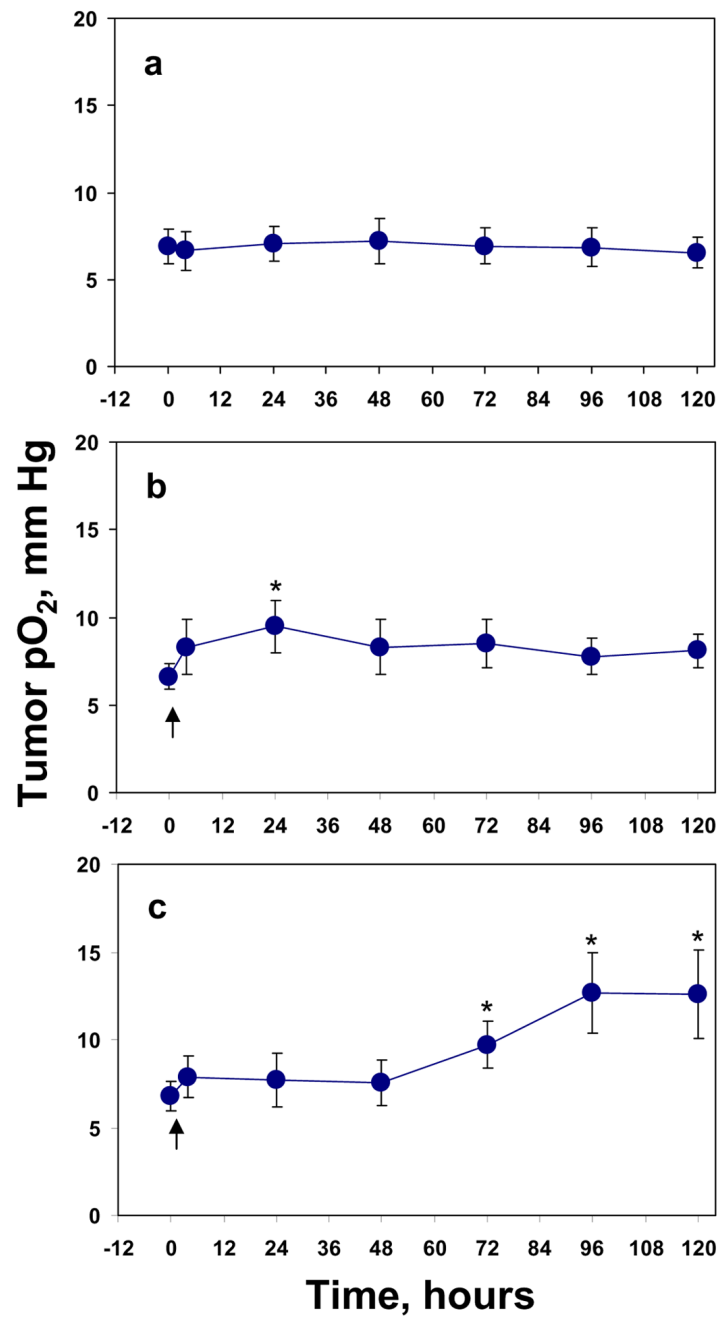
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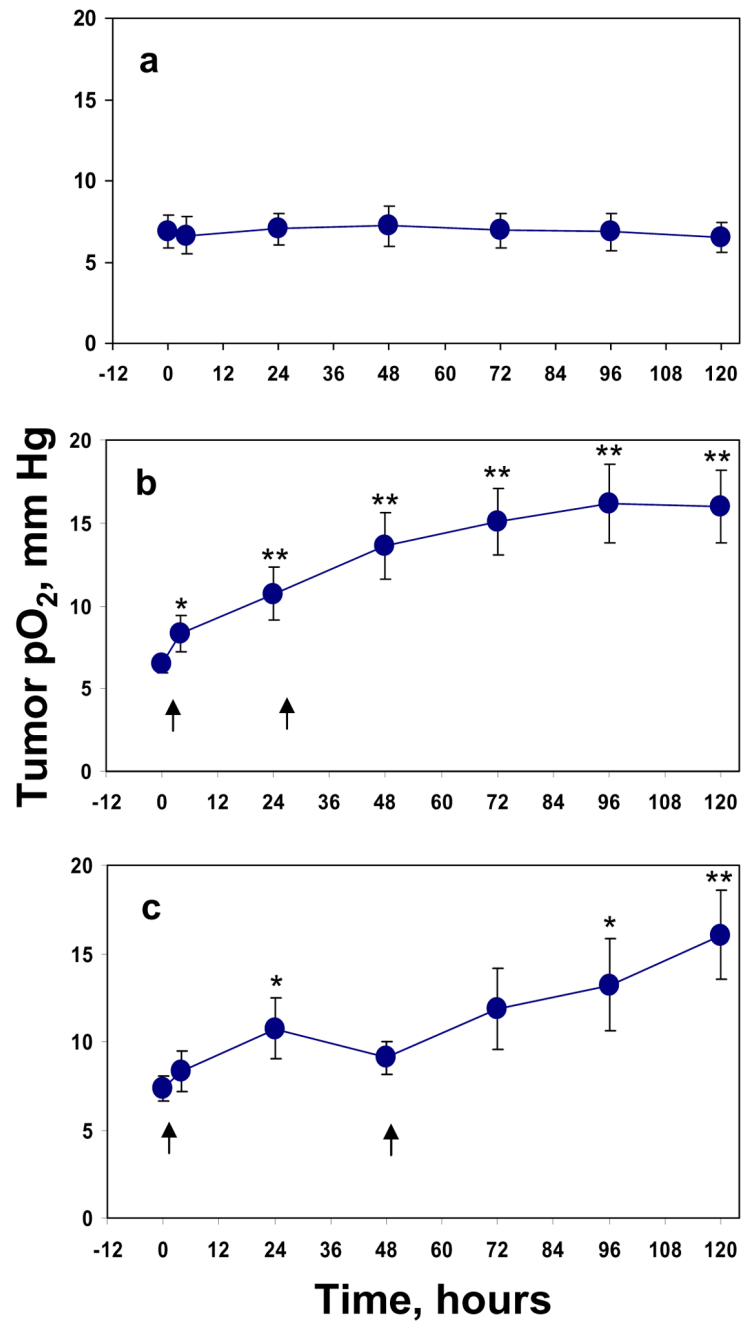
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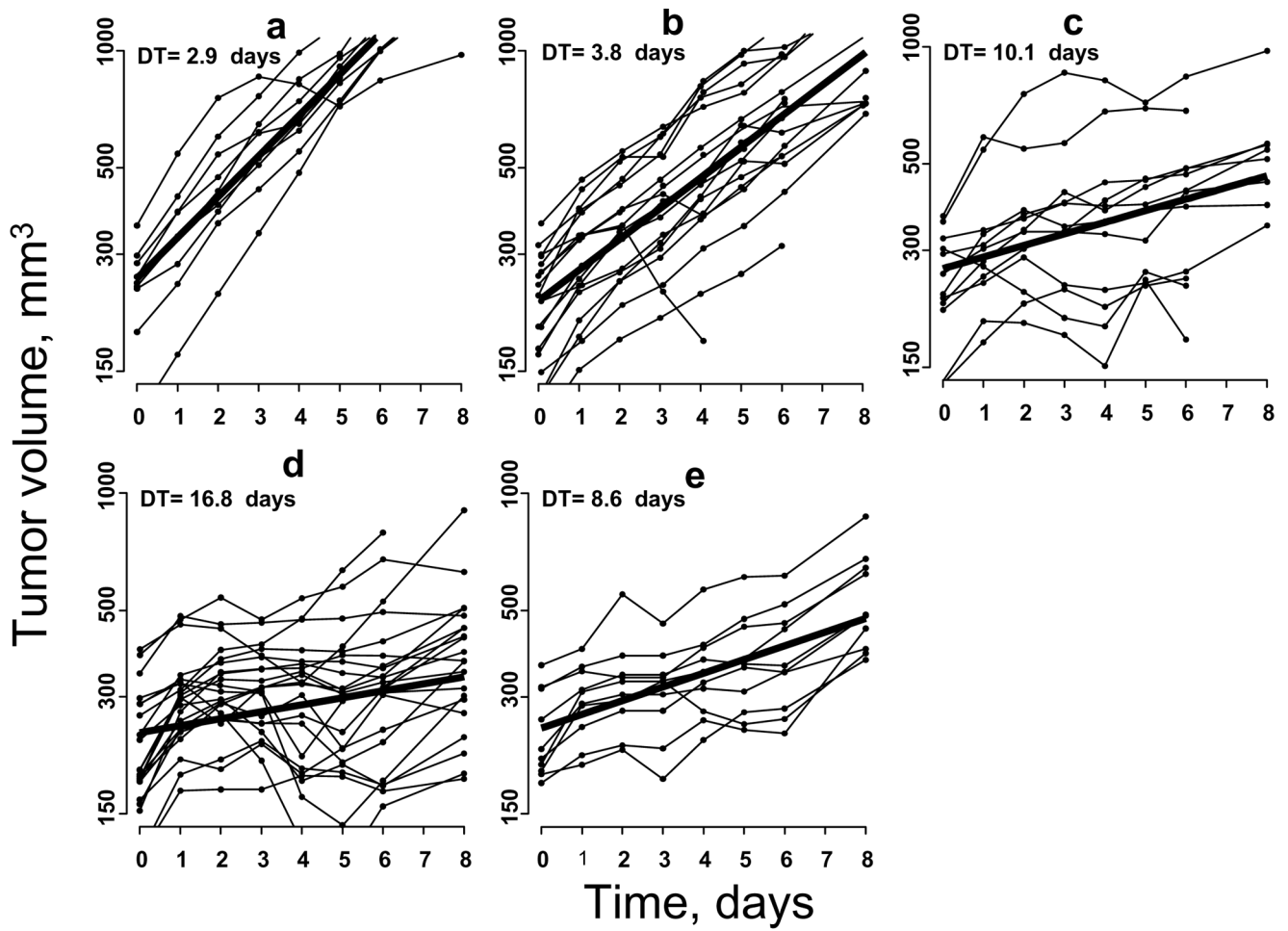


**Figure 1.** The change in average RIF-1 tumor pO<sub>2</sub> before and after a single or fractionated radiation. (a) 0 Gy, n = 18, (b) 10 Gy, n = 24–32, (c) 20 Gy, n = 18. n is the total number of EPR probes in the tumors. Arrows indicate the time of irradiation. \* p < 0.05, compared with baseline tumor pO<sub>2</sub> in the same group.



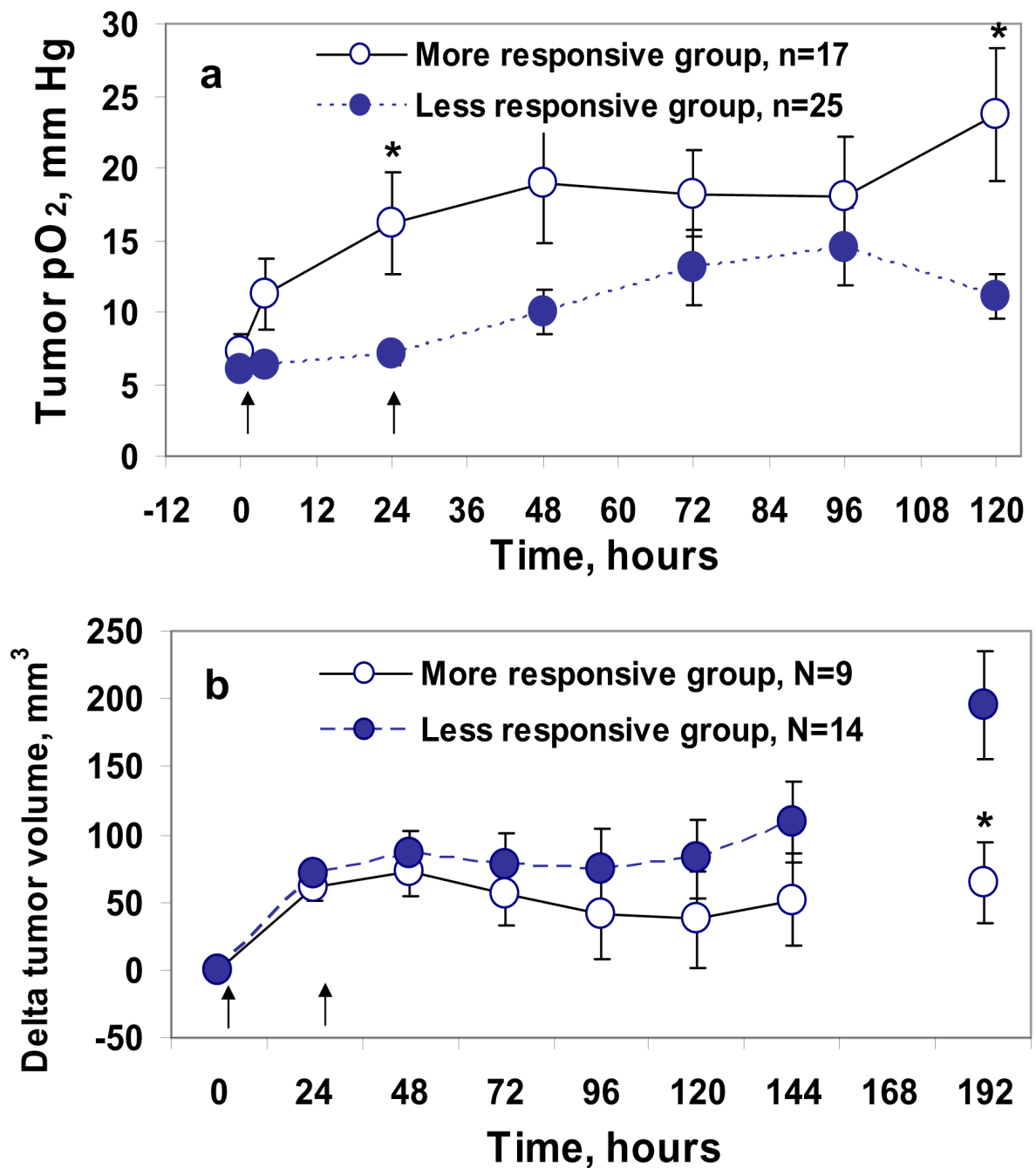
**Figure 2.**

The changes in average RIF-1 tumor pO<sub>2</sub> before and after split dose radiation. (a) 0 Gy, n = 18, (b) 10 Gy +10 Gy at 0/24 hr, n = 42, (c) 10 Gy +10 Gy at 0/48 hr, n = 19. Arrows indicate the time of irradiation. \* p < 0.05, \*\* p < 0.01, compared with baseline tumor pO<sub>2</sub> in the same group (paired t-test).



**Figure 3.**

The change in RIF-1 tumor volume in (a) 0 Gy, N = 9, (b) 10 Gy, N = 16, (c) 20 Gy, N = 9, (d) 10 Gy + 10 Gy at 0/24 hr, N = 24, (e) 10 Gy + 10 Gy at 0/48 hr, N = 10. N is the number of animals with RIF-1 tumors in each group. Bold line indicates the mean tumor volume. Exponential mixed modeling is used to determine the DT (23,24).



**Figure 4.** Tumor pO<sub>2</sub> (a) and tumor growth inhibition (b) of RIF-1 tumors before and after 10 Gy × 2 radiations. The interval between the doses is determined by the time course of changes in mean tumor pO<sub>2</sub> as described in the results section. Treatment groups were: (●) less responsive group (tumor pO<sub>2</sub> < 50 % of baseline), (○) more responsive group (tumor pO<sub>2</sub> ≥ 50 % of baseline). \* p < 0.05, compared with less responsive group (unpaired t-test). n is the number of LiPc implants in each group, N is the number of animals in each group.

**Table 1**  
Effect of radiation on RIF-1 tumor volume over days in control and irradiated groups

Tumor volume, mm <sup>3</sup>	G	N	Time, hours						
			0	24	48	72	96	120	144
A	9	250±23	95±15	216±31	341±33	457±39	628±47	752±146	1290±245
B	16	214±18	75±8	134±14 <sup>A</sup>	185±22 <sup>A</sup>	277±39 <sup>A</sup>	400±40 <sup>A</sup>	494±44 <sup>A</sup>	785±245 <sup>A</sup>
C	9	239±25	53±23	75±22 <sup>A</sup>	85±31 <sup>A</sup>	98±40 <sup>A</sup>	135±36 <sup>A</sup>	149±41 <sup>A</sup>	245±38 <sup>A</sup>
D	24	220±26	75±7	91±11 <sup>A,b</sup>	89±14 <sup>Aa,b</sup>	76±21 <sup>Aa,b</sup>	82±24 <sup>Aa,b</sup>	115±27 <sup>A,ab</sup>	144±31 <sup>A,ab,E</sup>
E	10	245±21	52±9	62±16 <sup>A</sup>	73±10 <sup>A</sup>	87±17 <sup>A</sup>	108±23 <sup>A</sup>	125±24 <sup>A</sup>	276±36 <sup>A</sup>

*Abbreviations:* G = groups. A, control group (sham-irradiated on day 0); B, single dose of 10 Gy radiation group (10 Gy on day 0); C, single dose of 20 Gy radiation group (20 Gy on day 0); D, 20 Gy oxygenated group (10Gy on day 0 and 10 Gy at 24 hr post first dose); E, 20 Gy hypoxic group (10Gy on day 0 and 10 Gy at 48 hr post first dose). N = numbers of animals. The values for time 0 are mean baseline absolute tumor volumes prior to the irradiation, while the values at other time points are relative mean changes in tumor volumes post irradiation. Differences between groups at the different time points were evaluated with unpaired t-test.

<sup>A</sup> p < 0.05,

<sup>a</sup> p < 0.01, compared with group A;

<sup>b</sup> p < 0.01, compared with group B;

<sup>E</sup> p < 0.05, compared with group E.