Chapter 53
Radiation Oxygen Biology with Pulse Electron Paramagnetic Resonance Imaging in Animal Tumors

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Abstract The reduced oxygen in tumors (hypoxia) generates radiation resistance and limits tumor control probability (TCP) at radiation doses without significant normal tissue complication. Modern radiation therapy delivery with intensity-modulated radiation therapy (IMRT) enables complex, high-dose gradient patterns, which avoid sensitive human tissues and organs. EPR oxygen images may allow selection of more resistant parts of a tumor to which to deliver more radiation dose to enhance TCP. EPR O_2 images are obtained using injected narrow-line, low relaxation rate trityl spin probes that enable pulse radiofrequency EPR O_2 images of tumors in the legs of mice, rats, and rabbits, the latter exceeding 4 cm in size. Low relaxation rates of trityls have enabled novel T_1-, rather than T_2-, based oximetry, which provides near absolute pO_2 imaging. Tomographic image formation and filtered back projection reconstruction are used to generate these images with fixed, linear stepped gradients. Images obtained both with T_2 and T_1 oximetric images have demonstrated the complex in vivo mechanism explaining the unexpected efficacy of TNFerade, a radiation-inducible adenoviral construct to locally produce TNF-induced vascular as well as radiation damage [1, 2]. The unexpected efficacy of large-dose radiation fractions is seen to be due to an interaction between host microvasculature and tumor cells producing a prompt (15 min) postradiation hypoxia, paralyzing tumor cell repair, and sensitizing tumors. Finally, cure of tumors treated to a single 50 % control dose shows a significant dependence on EPR

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O_2 image hypoxic fractions, best shown with the fraction of voxels less than 10 Torr (HF10). We show that these O_2 images provide a quantitative basis for measuring tumor and normal tissue response to abnormally low O_2 levels. Measurements of vascular endothelial growth factor (VEGF) production in a specific syngeneic mouse fibrosarcoma, FSa versus fraction of tissue voxels with pO_2 less than 10 Torr, produced a slope of 0.14 pg VEGF protein/mg total protein/% HF10. We argue that this quantification may be diagnostic of tumor versus normal tissue, and it may be etiologic in the development of malignancy.

53.1 Introduction

There are at least two reasons why images of local pO_2 in living animal tissues might be of general interest. The first is the importance of lack of oxygen in a large number of disease processes, including myocardial infarction, stroke, and cancer. The second reason for interest in local pO_2 oxygen images lies in the insight, in vivo, that such images may provide between an environmental stimulus or condition and the genetic response to it at the tissue and organismal level.

Hypoxia in cancer has been associated with resistance to genotoxic cancer treatments. Hypoxic resistance to radiation has been known for over a century, starting with the historic observations of Schwarz in 1909 [3]. The systematics of this evolved in the 1920s and 1930s with the work of Holthusen [4], as well as Gray, Conger, and others in the 1940s and 1950s [5], the quantification of the effect in cellular systems [6], and the appreciation of its applicability to human cancers, in particular the more recent work by Vaupel et al. [7–10].

The molecular biology of genetically induced cellular signalling has been the focus of most biologists for the past five decades [11]. Over the past two decades, the homeostatic centrality of the maintenance of adequate molecular oxygen has been recognized in the role of hypoxia-inducible factor, HIF, a master regulator of organismal signalling response to oxygen inadequacy [12]. The information leading to these conclusions has been obtained on either the cellular level or on the whole animal level. Heterogeneity of oxygen levels has not been addressed, particularly in tumors. The heterogeneity of tumor pO_2, shown in EPR O_2 images [13, 14] as well as needle electrode measurements of tumors, e.g., [15], requires the use of images to fully elucidate the relationship between the stimulating condition, hypoxia, and the genetic response.

We argue that in vivo images provide a robust means by which quantitative assessment of the unperturbed local oxygen environment can be made and then registered with the molecular signals that respond to the environment. This will form the basis of a quantitative stimulus–response model of the molecular response to the environment. Local abnormalities in this stimulus–response relationship may be etiologic in disease process. Such abnormalities may be based in abnormal transcription, as is assumed by most molecular biologists. This ultimately assumes that the only active agent in disease process is the genome. However, we argue that

this is a simplified model, and it ignores a number of aspects of tissue and cell environment that can be disrupted and contribute to disease process. For example, vessel dysfunction, particularly microvessel dysfunction, can interfere with intercellular communication as part of paracrine and endocrine signalling processes. Intracellular protein glycation markedly affects endothelial function [16] and can have a similar effect on autocrine signalling and carcinogenesis [17–19]. These mechanisms may interfere with diffusion or translocation of signals. They will then change the relationship between the environmental trigger and the molecular response. They will change, for example, the dose–response relationship between local concentrations of oxygen and the HIF1-associated signal response elements. By concentrating solely on the relationship between hypoxia and HIF1 response and disregarding other possible environmental factors, we can approximate such a response as a simple linear function. In this simplified model, HIF1-associated signal (e.g., VEGF) would increase linearly as the pO_2 diminishes. There may be a normal slope or intercept in this relationship. Disease process may be reflected in alterations of the slope or intercept seen in the correlation between pO_2 and signal.

53.2 Methods

Electron paramagnetic resonance (EPR) O_2 imaging (**EPROI)** gives quantitative localized pO_2 images of various tumors in syngeneic mice, rats, and rabbits [20–22]. The oxygen broadening of narrow EPR spectral lines, or, equivalently, the increase in relaxation rates of electron magnetization, reports the pO_2 with 1–2 Torr resolution in image voxels as small as 1 mm^3 [13, 23]. We have found remarkable freedom from toxicity in 283 mice injected with the pO_2 reporter molecule OX063 (GE Healthcare) used to acquire the data reported here. OX063 is extracellular in its distribution and is rapidly cleared from the body through renal excretion with a half-life of ~5 min. It appears to be selectively retained in tumors with a half-life of ~30 min. This bodes well for eventual application for human subjects. OX063 and the partially deuterated O_2 reporter $OX063_{d24}$, also known as OX071, have very limited dependence on viscosity. We have found that, by using pulse sequences that image the longitudinal relaxation rate, R_1 ($R_1 = 1/T_1$, where T_1 is the longitudinal relaxation time), self-relaxation of the trityl spin probe is reduced to well within the 1 Torr uncertainties of our image voxel pO_2 values. EPROI:

1. Correlates point by point with Oxylite measurements [13]
2. Significantly sharpens the tumor cure prediction (along with dose in bivariate analysis) [20]
3. Independently distinguishes sensitive from resistant animal tumors treated with a single-dose magnitude, the 50 % tumor control dose, TCD_{50}, in two tumor types
4. Provides in vivo measurement of the rapid postradiation hypoxia induced via vascular apoptosis in response to large fraction radiation [24]

Other in vivo oxygen or hypoxia imaging modalities include ^{19}F MRI and ^{18}F-misonidazole and ^{64}Cu-ATSM positron emission tomography (PET). PET can be used for human studies. ^{19}F MRI is presently used only in animal studies. Neither of these other imaging modalities or other available in vivo animal O_2 images provide such a combination of accuracy in the voxel pO_2, lack of confounding biologic variability, and low level of invasiveness and toxicity.

Hypoxia in EPROI has been locally correlated with concentrations of the hypoxia protein vascular endothelial growth factor (VEGF) in biopsies stereotactically registered with the pO_2 images. These methods of registration and VEGF quantification have been described previously [21].

53.3 Results

In Fig. 53.1, obtained with natural isotopic abundance OX063 as the oxygen reporter, we show the correlation between the fraction of voxels with pO_2 less than 10 Torr (HF10) and the concentration of VEGF in biopsies obtained from 12 fibrosarcomas grown intramuscularly in the legs of C3H mice to volumes of 0.5 ml (10 mm equivalent diameters), as previously described [21]. For each biopsy, the absolute concentration of VEGF was determined from the specimen. There were ~75 pO_2 image voxels to obtain statistics from for each biopsy sample. This allowed us to compute the HF10, which appeared to be the most reliable statistic among mean pO_2, median pO_2, HF10, HF5, and HF2.5. Uncertainties in the HF10 and in

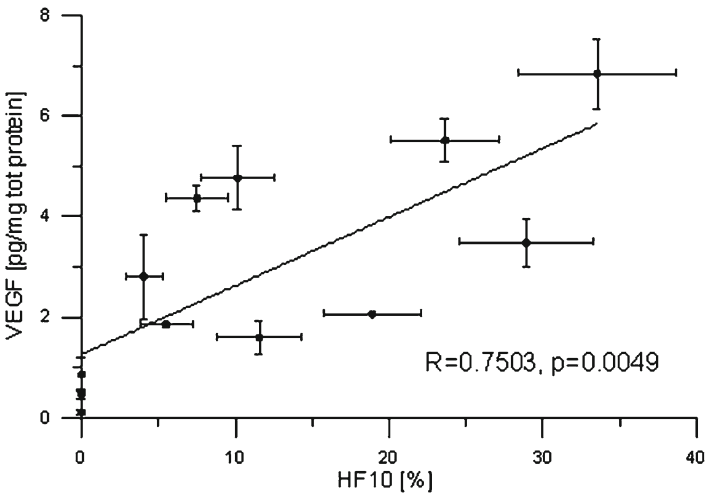

Fig. 53.1 Correlation between hypoxia, defined by the percentage of voxels in the biopsy with pO_2 less than 10 Torr as determined from the EPR oxygen image, and the VEGF concentration in pictograms per microgram of total protein

the VEGF concentration from each sample are shown in Fig. 53.1. The Pearson product–moment correlation coefficient, R = 0.75, provides a basis for establishing the fraction of variation in the samples that is due to true interdependence. The regression slope of the tumor tissue response is 0.14 pg VEGF/mg tumor tissue/%HF10. This is a measure, although undoubtedly a simplified one, of the signal response by tumor cells to produce more vascular endothelial cells in response to local hypoxia, as measured by percentage HF10. The heterogeneity of tumor pO_2, as shown in EPROI [13, 14] as well as needle electrode measurements of tumors [15], requires the use of images to define local tumor pO_2 and HF10. Because of the large variation of pO_2 and HF10 between the 20 μl biopsy volumes obtained in the study (typically 2 per tumor, one high and one low pO_2), a relatively small number of samples achieved significance, as seen in Fig. 53.1.

53.4 Discussion and Conclusions

This correlation between stereotactic biopsy-derived VEGF concentrations and biopsy sample HF10 values from registered EPROI provided statistically significant correlation between the two values. We argue that this slope, 0.14 pg VEGF/mg tumor tissue/%HF10, is a measure of tissue and tumor response to hypoxia. Although normal tissue measurements remain to be generated, we argue that this may be not only different, and a diagnostic of the difference between tumor and normal tissue, but that it may be etiologic in the development of malignancy. The dysfunctional chaos of tumor growth may stimulate either a reduced or an enhanced tissue response. EPROI may provide the basis for quantification of this response and introduce a new, quantitative aspect for evaluating tumor versus normal tissue response to the hypoxic environment.

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References

1. Hallahan DE, Mauceri HJ, Seung LP et al (1995) Spatial and temporal control of gene therapy using ionizing radiation. Nat Med 1(8):786–791
2. Haney CR, Parasca AD, Fan X et al (2009) Characterization of response to radiation mediated gene therapy by means of multimodality imaging. Magn Reson Med 62(2):348–356
3. Schwarz G (1909) Über Desensibilisierung gegen Röntgen- und Radiumstrahlen. Munchner Medizinische Wochenschrift 56:1217–1218
4. Holthusen H (1921) Beitrage zur biologie der strahlenwirkung. Pflugers Arch 187:1–24
5. Gray LH, Conger AD, Ebert M, Hornsey S, Scott OC (1953) The concentration of oxygen dissolved in tissues at the time of irradiation as a factor in radiotherapy. Br J Radiol 26(312): 638–648
6. Howard-Flanders P (1958) Physical and chemical mechanisms in the injury of cells by ionizing radiation. In: Lawrence JH, Tobias CA (eds) Advances in biology and medical physics, vol 13. Academic, New York, pp 553–603

7. Thomlinson RH, Gray LH (1955) The histological structure of some human lung cancers and the possible implications for radiotherapy. Br J Radiol 9(4):539–563
8. Gatenby RA, Kessler HB, Rosenblum JS et al (1988) Oxygen distribution in squamous cell carcinoma metastases and its relationship to outcome of radiation therapy. Int J Radiat Oncol Biol Phys 14(5):831–838
9. Hockel M, Schlenger K, Mitze M, Schäffer U, Vaupel P (1996) Hypoxia and radiation response in human tumors. Semin Radiat Oncol 6(1):3–9
10. Brizel DM, Scully SP, Harrelson JM et al (1996) Tumor oxygenation predicts for the likelihood of distant metastases in human soft tissue sarcoma. Cancer Res 56(5):941–943
11. Alberts B, Johnson A, Lewis J et al (2008) Molecular biology of the cell, 5th edn. Garland Science, New York
12. Semenza GL (1988) Hypoxia-inducible factor 1: master regulator of O_2 homeostasis. Curr Opin Genet Dev 8(5):588–594
13. Elas M, Ahn KH, Parasca A et al (2006) Electron paramagnetic resonance oxygen images correlate spatially and quantitatively with oxylite oxygen measurements. Clin Cancer Res 12(14 Pt 1):4209–4217
14. Elas M, Williams BB, Parasca A et al (2003) Quantitative tumor oxymetric images from 4D electron paramagnetic resonance imaging (EPRI): methodology and comparison with blood oxygen level-dependent (BOLD) MRI. Magn Reson Med 49(4):682–691
15. Kallinowski F, Zander R, Hoeckel M, Vaupel P (1990) Tumor tissue oxygenation as evaluated by computerized-pO_2-histography. Int J Radiat Oncol Biol Phys 19(4):953–961
16. Wautier JL, Schmidt AM (2004) Protein glycation: a firm link to endothelial cell dysfunction. Circ Res 95(3):233–238
17. Tafani M, Schito L, Pellegrini L et al (2011) Hypoxia-increased RAGE and P2X7R expression regulates tumor cell invasion through phosphorylation of Erk1/2 and Akt and nuclear translocation of NF-kappa B. Carcinogenesis 32(8):1167–1175
18. Kang R, Loux T, Tang D et al (2012) The expression of the receptor for advanced glycation endproducts (RAGE) is permissive for early pancreatic neoplasia. Proc Natl Acad Sci USA 109(18):7031–7036
19. Rojas A, Figueroa H, Morales E (2010) Fueling inflammation at tumor microenvironment: the role of multiligand/rage axis. Carcinogenesis 31(3):334–341
20. Elas M, Bell R, Hleihel D et al (2008) Electron paramagnetic resonance oxygen image hypoxic fraction plus radiation dose strongly correlates with tumor cure in FSa fibrosarcomas. Int J Radiat Oncol Biol Phys 71(2):542–549
21. Elas M, Hleihel D, Barth ED et al (2011) Where it's at really matters: in situ in vivo vascular endothelial growth factor spatially correlates with electron paramagnetic resonance pO_2 images in tumors of living mice. Mol Imaging Biol 13(6):1107–1113
22. Epel B, Haney CR, Hleihel D, Wardrip C, Barth ED, Halpern HJ (2010) Electron paramagnetic resonance oxygen imaging of a rabbit tumor using localized spin probe delivery. Med Phys 37(6):2553–2559
23. Epel B, Sundramoorthy SV, Mailer C, Halpern HJ (2008) A versatile high speed 250-MHz pulse imager for biomedical applications. Concept Magn Reson Part B Magn Reson Eng 33B(3):163–176
24. Thin TH, García-Barros M, Fuller J, et al (in preparation) Epigenetic regulation of homologous recombination by microvascular dysfunction controls tumor cure by single dose radiation. Cell