Brca1/p53 Deficient Mouse Breast Tumor Hemodynamics During Hyperoxic Respiratory Challenge Monitored by A Novel Wide-field Functional Imaging (WiFI) System

Austin Moy*[1,2], Jae G. Kim[1], Eva YHP Lee[3], Bruce Tromberg[1,2], Albert Cerussi[1], Bernard Choi[1,2]
[1]Beckman Laser Institute and Medical Clinic, 1002 Health Sciences Rd., Irvine, CA USA 92612;
[2]Dept. of Biomedical Engineering, University of California, Irvine, Irvine, CA USA 92697;
[3]Dept. of Developmental and Cell Biology and Dept. of Biological Chemistry, University of California, Irvine, Irvine, CA USA 92697

ABSTRACT

Current imaging modalities allow precise visualization of tumors but do not enable quantitative characterization of the tumor metabolic state. Such quantitative information would enhance our understanding of tumor progression and response to treatment, and to our overall understanding of tumor biology. To address this problem, we have developed a wide-field functional imaging (WiFI) instrument which combines two optical imaging modalities, spatially modulated imaging (MI) and laser speckle imaging (LSI). Our current WiFI imaging protocol consists of multispectral imaging in the near infrared (650-980 nm) spectrum, over a wide (7 cm x 5 cm) field of view. Using MI, the spatially-resolved reflectance of sinusoidal patterns projected onto the tissue is assessed, and optical properties of the tissue are estimated using a Monte Carlo model. From the spatial maps of local absorption and reduced scattering coefficients, tissue composition information is extracted in the form of oxy-, deoxy-, and total hemoglobin concentrations, and percentage of lipid and water. Using LSI, the reflectance of a 785 nm laser speckle pattern on the tissue is acquired and analyzed to compute maps of blood perfusion in the tissue. Tissue metabolism state is estimated from the values of blood perfusion, volume and oxygenation state. We currently are employing the WiFI instrument to study tumor development in a BRCA1/p53 deficient mice breast tumor model. The animals are monitored with WiFI during hyperoxic respiratory challenge. At present, four tumors have been measured with WiFI, and preliminary data suggest that tumor metabolic changes during hyperoxic respiratory challenge can be determined.

Keywords: Tissue optics, speckle, cancer, metabolism, transgenic, laser Doppler perfusion imaging

1. INTRODUCTION

Cells require readily available oxygen and nutrients, such as growth factors and amino acids, to survive. These components are delivered to cells by the blood via the microvasculature. Normal microvasculature is composed of mature vessels and maintained by pro- and anti-angiogenic molecules. Tumor microvasculature, by contrast, is structurally and functionally abnormal and is characterized by tortuous, dilated and saccular blood vessels that are poorly organized and have increased permeability. This results in a vascular network that has spatial and temporal heterogeneity and greatly inhibits the delivery of oxygen and macromolecule nutrients. Finally, poor oxygen delivery creates a hypoxic environment within the tumor. These hallmark characteristics of solid tumors act in concert to limit the delivery and effectiveness of both cytotoxic and molecular targeted therapies[1].

Hyperoxic respiratory challenges have been applied to enhance the efficacy of cancer treatments such as radiotherapy by increasing tumor oxygenation[2]. Due to the abnormal state of tumor vasculature, tumors show a different response from normal tissue to respiratory challenges. In this study, we monitored changes in tumor hemodynamic parameters such as blood oxygenation, blood volume, and blood perfusion during oxygen gas intervention using a novel wide field functional imaging (WiFI) system.

*amoy@uci.edu; phone: 949-824-3054; choi.bli.uci.edu

Optical Tomography and Spectroscopy of Tissue VIII, edited by Bruce J. Tromberg, Arjun G. Yodh, Mamoru Tamura, Eva M. Sevick-Muraca, Robert R. Alfano, Proc. of SPIE Vol. 7174
71742L · © 2009 SPIE · CCC code: 1605-7422/09/$18 · doi: 10.1117/12.823745

The WiFI system consists of two components: spatially modulated imaging (MI) and laser speckle imaging (LSI). MI provides information about the biochemical composition of the tissue in question while LSI provides information about blood perfusion to the tissue.

2. MATERIALS AND METHODS

2.1 Instrumentation

2.1.1 Modulated imaging

MI makes use of spatially varying patterns (i.e. sinusoidal) of broadband light that are projected onto the tissue. These patterns are projected at different intervals, or spatial frequencies. Each of these spatial frequencies is then shifted by a certain spatial offset, or phase. Depending on the spatial frequency, different depths of tissue can be interrogated; low frequency patterns probe deeper into the tissue (maximum of 5 mm) while high frequency patterns probe shallower depths of tissue. Near IR reflectance images of the tissue are then acquired from the light projected at each of the different frequency and phase combinations onto the tissue using a camera equipped with a tunable liquid crystal filter set for the NIR regime. Optical properties (absorption and reduced scattering) of the tissue can be determined by demodulating the various frequency/phase images. From these optical properties, tissue chromophore concentrations can be calculated using a diffusion-based model of light propagation in tissue[3]. The tissue chromophores include oxyhemoglobin (HbO_2), deoxyhemoglobin (Hb), total hemoglobin (THb), lipid, and water. Based on HbO_2 and Hb values, we compute oxygen saturation (S_tO_2).

2.1.2 Laser speckle imaging

LSI utilizes temporal changes in the speckle pattern of coherent laser light to detect changes in blood perfusion. We irradiate tissue with a low-power diode laser (785 nm) and reflectance images of the speckle pattern are acquired. The reflectance, or raw speckle, image is then converted to a speckle contrast image with a sliding window operator. Finally, a speckle flow index (SFI) map, computed from the contrast image, provides tissue perfusion information. We have employed LSI to study blood flow dynamics of the rodent dorsal window chamber model[4] as well as application in the clinic in assessing port wine stain treatment[5].

2.2 Animal model

A p53/Brca1 knockout murine model developed by Dr. Eva Lee's lab was used. This model develops spontaneously occurring transgenic breast tumors[6]. Animals had tumors of sufficient size that were observed by visual inspection and were between 8-12 months of age at the start of the study.

2.3 Experimental protocol

Animal subjects were continuously measured using WiFI during medical grade air inspiration and subsequent 100% oxygen challenge. Medical air inspiration duration was eight min in duration and subsequent 100% oxygen inspiration was 20 min. The total measurement time was 28 minutes.

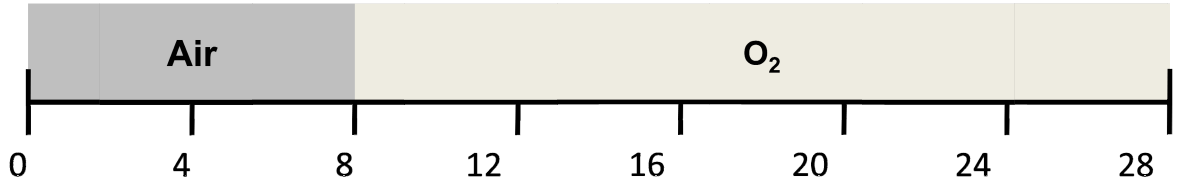


Figure 1: Diagram of experimental protocol detailing inspiration time of each gas

3. DATA

Below is a table of the changes in the pertinent tissue chromophores HbO_2, Hb, and THb, and functional parameters S_tO_2, and SFI, during a respiratory challenge.

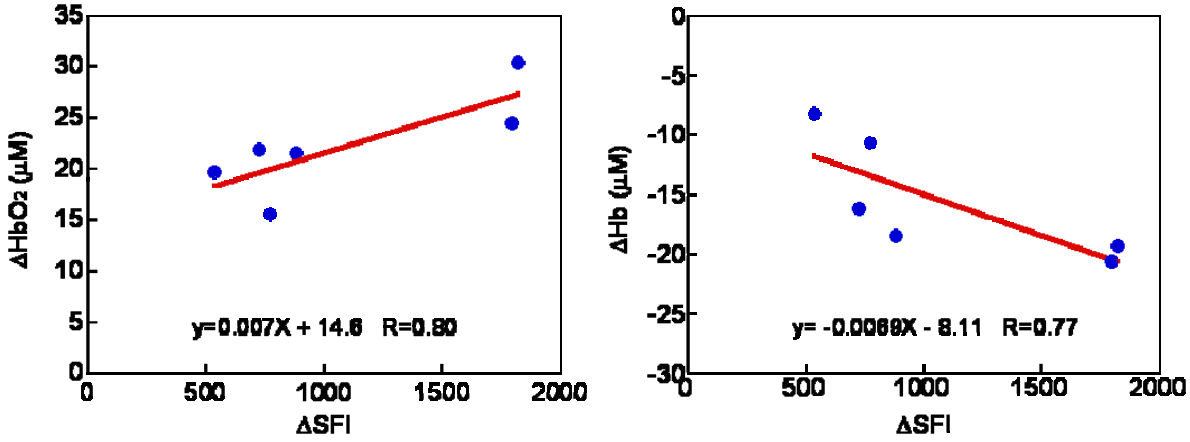
Table 1. Change in tissue chromophores and functional parameters measured with WiFI during a hyperoxic challenge. Data are from six separate measurements on different days of study.

Day	**ΔHbO$_2$** (μM)	**ΔHb** (μM)	**ΔTHb** (μM)	**ΔS$_t$O$_2$** (%)	**ΔSFI**
12	24.43	-20.627	3.8	12.169	1794
17	19.7	-8.23	11.46	5.741	535
19	30.45	-19.296	11.15	11.599	1818
24	21.58	-18.438	3.14	10.239	880
26	21.84	-16.172	5.07	9.169	723
28	15.5	-10.678	4.82	6.639	772

The mammary metabolic rate of oxygen ($MMRO_2$)[7] is a parameter that we used to describe the oxygen metabolism of the tumor during oxygen challenge. It is based on the total hemoglobin concentration, deoxyhemoglobin concentration, and blood perfusion values measured during air and oxygen inspiration. The parameter is defined as:

$$MMRO_2(O_2 / Air) = \frac{Hb(O_2)}{Hb(Air)} \times \frac{THb(Air)}{THb(O_2)} \times \frac{SFI(O_2)}{SFI(Air)}$$

Below are plots of the change in each of the tissue chromophores against the change in SFI value. These plots give information about the relationship between the change in blood perfusion and the change in chromophore concentration.



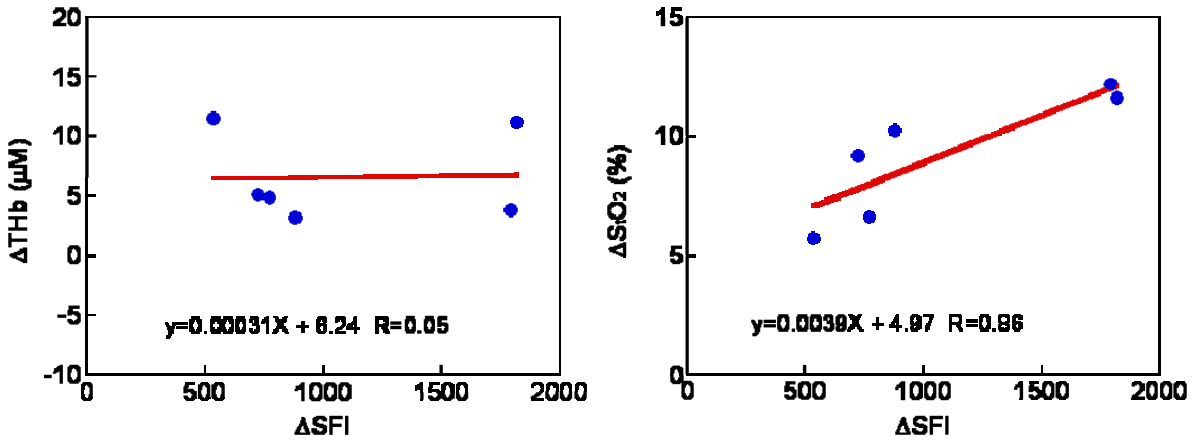


Figure 2: Plots of the changes in oxyhemoglobin (HbO_2), deoxyhemoglobin (Hb), total hemoglobin (THb), and oxygen saturation (S_tO_2) against the change in speckle flow index (SFI)

Measurement of the tissue chromophore values during air inspiration and oxygen challenge allows for calculation of the $MMRO_2$ during air inspiration and oxygen challenge. Figure 3 below shows the average change over six measurements in HbO_2, Hb, THb, S_tO_2, SFI, and $MMRO_2$ during air inspiration and oxygen challenge.

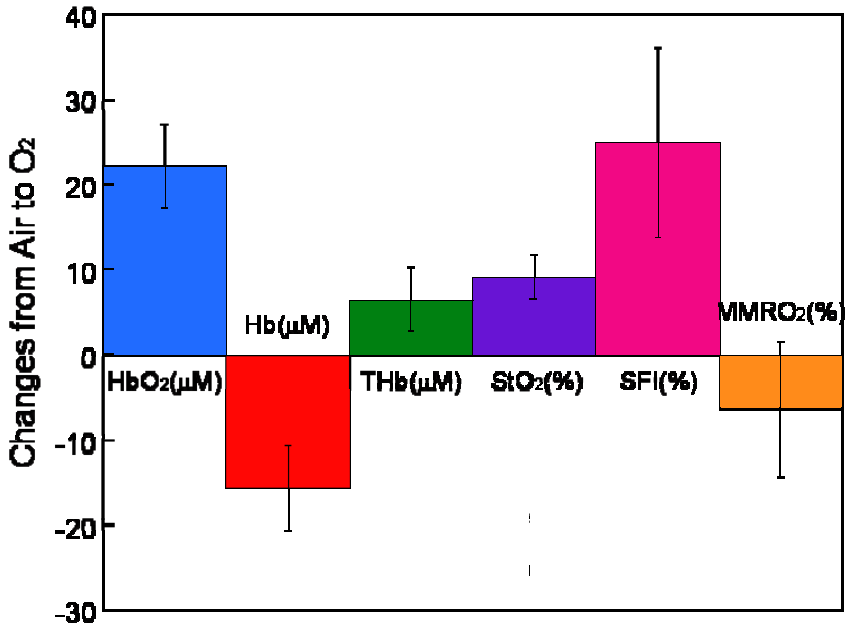


Figure 3: Bar graph of the change in tissue chromophores, speckle flow index, and mammary metabolic rate of oxygen

4. DISCUSSION

In general, an increase in the change of HbO_2 and S_tO_2 as well as a decrease in the change Hb is observed during oxygen challenge, as seen in both Figure 2 and Figure 3, corresponding to an increase in the change in blood perfusion (SFI) and decrease in the change of $MMRO_2$. In addition, there is a strong positive correlation between the change in HbO_2 and S_tO_2 and change in blood perfusion during oxygen challenge. Correspondingly, there is a strong negative correlation between decreases in the change in Hb concentration and increases in the change in blood perfusion. An interesting observation is that there does not appear to be any correlation between changes in total hemoglobin concentration and

changes in SFI values. This observation bears further investigation as it potentially may provide insight into the relationship between blood perfusion and blood content.

We have shown that our novel WiFI system has the potential to monitor tumor metabolic changes during hyperoxic respiratory challenge. The information obtained from this study can potentially give further insight into relationships between blood perfusion and corresponding changes in tissue chromophore concentration. Future work includes continuing these measurements on more tumors and determining the interplay between each of these tumor metabolic markers.

REFERENCES

1. Jain RK, "Normalizing Tumor Vasculature with Anti-Angiogenic Therapy: A New Paradigm for Combination Therapy," Nature Medicine 7, 987-989 (2001).

2. Hou H, Lariviere JP, Demidenko E, Gladstone D, Swartz H, Khan N, "Repeated tumor pO(2) measurements by multi-site EPR oximetry as a prognostic marker for enhanced therapeutic efficacy of fractionated radiotherapy," Radiotherapy and Oncology (2008) [Epub ahead of print].

3. Cuccia DJ, Bevilacqua F, Durkin AJ, Tromberg BJ, "Modulated imaging: quanititative analysis and tomography of turbid media in the spatial-frequency domain," Optics Letters 30(11), 1354-1356 (2005).

4. Choi B, Kang NM, Nelson JS, "Laser speckle imaging for monitoring blood flow dynamics in the in vivo rodent dorsal skin fold model," Microvascular Research 68(2), 143-146 (2004).

5. Huang YC, Ringold TL, Nelson JS, Choi B, "Noninvasive blood flow imaging for real-time feedback during laser therapy of port wine stain birthmarks," Lasers in Surgery and Medicine 40(3), 167-173 (2008).

6. Shafee N, Smith CR, Wei S, Kim Y, Mills GB, Hortobagyi GN, Stanbridge EJ, Lee EY, "Cancer stem cells contribute to cisplatin resistance in Brca1/p53-mediated mouse mammary tumors, "Cancer Research 68(9), 3243-50 (2008).

7. Zhou C, Choe R, Shah N, Durduran T, Yu G, Durkin A, Hsiang D, Mehta R, Butler J, Cerussi A, Tromberg BJ, Yodh AG., "Diffuse optical monitoring of blood flow and oxygenation in human breast cancer during early stages of neoadjuvant chemotherapy," Journal of Biomedical Optics 12(5), 051903 (2007).