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SPIE.

Event: SPIE BiOS, 2015, San Francisco, California, United States

Optical-resolution Photoacoustic Microscopy of the Metabolic Rate of Oxygen in a Mouse Renal Tumor Model

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ABSTRACT

We propose using noninvasive longitudinal optical-resolution photoacoustic microscopy (L-ORPAM) to quantify blood flow flux, oxygen saturation (sO_2), and thereby the metabolic rate of oxygen (MRO_2), for a renal tumor model in the same mouse over weeks to months. Experiments showed that the sO_2 difference between the artery and vein decreased greatly due to the arteriovenous shunting effect during tumor growth. Moreover, hypermetabolism was exhibited by an increase in MRO_2 .

Keywords: photoacoustic microscopy, optical-resolution, oxygen saturation, tumor, cancer, metabolic rate of oxygen

1. INTRODUCTION

The microcirculation supports nutrition for cell growth. For this reason, both the vascular network and hemodynamic change are required for tumor growth and thus play an important role in the progression of cancer. Understanding the corresponding metabolism change in response to vascular microcirculation change has become a new approach to monitor cancer therapy [1, 2].

Intravital microscopy (IVM), the gold standard in studying microcirculation, can observe a single vessel at high frame rates [3], revealing changes in vessel diameter [4]; moreover, red blood cell (RBC) flow can be observed via fluorescent-dye-labeled RBCs [5]. Nevertheless, IVM requires surgical preparation and transillumination to observe capillaries *in vivo*. Also, IVM lacks depth resolution for constructing three-dimensional (3-D) images of microvascular network morphology. Other full-field imaging modalities, including intrinsic optical imaging [6, 7], laser speckle imaging [7], laser Doppler imaging [8], and blood-oxygenation level-dependent functional magnetic resonance imaging (BOLD fMRI) [9], are effective in deep tissue imaging, but lack sufficient spatial resolution for microvascular imaging. Two-photon microscopy (TPM) and confocal microscopy can achieve high spatial resolution, are noninvasive, and can acquire 3-D volumetric microvascular morphology. However, their imaging contrast still relies on exogenous fluorescent agents that could possibly limit their applications in laboratory research [10]. Optical micro-angiography (OMAG), developed from Fourier domain optical coherence tomography (FD-OCT), has achieved intrinsic imaging of the microvascular network down to the capillary level [11]. However, OMAG encounters challenges in imaging functional

information, such as the oxygen saturation (sO_2) of hemoglobin, and its sensitivity is not yet sufficient to image a single blood cell.

Photoacoustic tomography (PAT), using optical excitation and ultrasonic detection, is a promising tool that creates multiscale images of living biological structures [12-19]. Optical resolution photoacoustic microscopy (OR-PAM), which evolved from PAT, offers multiple advantages. First, OR-PAM uses endogenous hemoglobin absorption contrast to image the microvasculature which supplies tissue with oxygen and nutrition. Second, tissue transparency is enhanced by the one-way ultrasonic path, since in biologic tissues acoustic scattering is less than optical scattering. Third, the high non-radiative quantum yield of hemoglobin (100% sensitivity) and the improved tissue transparency allow single-RBC-level imaging sensitivity. Finally, the noninvasive nature of OR-PAM enables repetitive imaging of hemodynamic activity at the capillary level in the same animal.

2. METHODS AND MATERIALS

2.1 OR-PAM

As shown in Fig. 1, the optical-resolution photoacoustic microscopy (OR-PAM) system uses a solid-state laser (INNOSLAB, Edgewave: 532 nm and 559 nm) for functional imaging [20]. The laser beam passes through an iris for spatial filtering, and then is focused by a condenser lens (LA1131, Thorlabs) to pass through a 50- μ m-diameter pinhole (P50C, Thorlabs). The pinhole offers effective spatial filtering for the fundamental-mode laser beam. The laser beam is then coupled into a single-mode fiber (LMA-10, NKT Photonics). The output laser beam from the fiber is collimated by a microscope objective (RMS4X, Thorlabs) and focused by another identical objective to a diffraction-limited optical focus of 2.04 μ m at 532 nm. The generated photoacoustic waves are reflected by a layer of silicone oil and then detected by an ultrasonic transducer (V214-BB-RM, Olympus-NDT). The optical-acoustic scanning head is moved by two linear motor stages (PLS-85, PI miCos) in the x-y plane and a linear stage (VT-21 S, PI miCos) in the z-direction.

Figure 1. Photoacoustic Microscopy. Schematic diagram of OR-PAM. ConL, condenser lens; ND, neutral density filter; FC, fiber collimator; SMF, single-mode fiber; BS, beam splitter; PD, photodiode; CorL, correction lens; RAP, right-angle prism; SO, silicone oil; RhP, rhomboid prism; UST, ultrasonic transducer.

2.2 Multi-parameter functional OR-PAM

PAM is capable of measuring multi-functional hemodynamic parameters to determine the metabolic rate of oxygen (MRO_2) [2]. Functional parameters, including vessel diameter, blood flow speed, total hemoglobin concentration,

oxygen saturation, and tissue volume, were monitored using OR-PAM. First, vascular structure was acquired by hemoglobin contrast, and then the vessel diameter was calculated from the cross-section perpendicular to the vessel centerline. Moreover, the ears volume was approximately calculated by integrating the voxels within the whole ear's anatomical structure. Second, total hemoglobin concentration was acquired at an isosbestic wavelength at 532 nm. Third, the blood flow speed was measured using the photoacoustic Doppler bandwidth broadening at 532 nm.[21] We used 4000 sequential A-lines to calculate the bandwidth broadening. Fourth, with dual-wavelength measurements (at 532 nm, an isosbestic wavelength of hemoglobin, and at 559 nm, a nonisosbestic wavelength of hemoglobin), OR-PAM acquired hemoglobin oxygen saturation (sO_2) at the single RBC level.[22]

The oxygen extraction fraction (OEF) is by definition[23]

$$OEF = (sO_{2artery} - sO_{2vein}) / (sO_{2artery}).$$

The blood volumetric flux rate (VFR) was calculated from the blood flow speed (v), vessel cross-section area (A), and diameter (r) over the selected vessel cross section:

$$VFR = \int v dA = \int v \times 2 \times \pi \times r \times dr.$$

The metabolic rate of oxygen (MRO₂) was calculated from the OEF, blood volumetric flux rate (VFR), ear weight ($W = (\text{ear volume}) \times (\text{density})$), and arteriolar oxygen concentration [O_2]_a [23] :

$$MRO_2 = OEF \times VFR \times [O_2]_a / W.$$

2.3 Ethical review of procedures

All experimental animal procedures were carried out in conformance with the laboratory animal protocol approved by the School of Medicine Animal Studies Committee of Washington University in St. Louis.

2.4 Tumor xenograft model

786-O renal cell carcinoma cells (1×10^6 cells in 10 μ L of RPMI basal medium, CRL-1932, ATCC, Manassas, VA) were injected subcutaneously into one ear of a 6–8 week old nude mouse (Hsd:Athymic Nude-Foxn 1NU, Harlan Co.).

2.5 Animal preparation for imaging

The nude mouse (Hsd:Athymic Nude-Foxn 1NU, Harlan Co.) was anesthetized with isoflurane and taped to a heating pad to maintain body temperature. The mouse ear was adhered to the plastic plate with ultrasound gel. The gel was also applied between the ear's surface and the polyethylene membrane.

3. RESULTS AND DISCUSSION

3.1 *In vivo* microvascular imaging

A 12 mm-by-12 mm region of a nude mouse ear was imaged *in vivo* by OR-PAM at the isosbestic wavelength 532 nm, as shown in Fig. 2.

3.2 *In vivo* longitudinal imaging of tumor hemodynamics

The high-resolution and image segmentation capability of OR-PAM allowed multi-parameter analysis of the vascular morphology, including a 3-D vessel network map (left column of each group (Fig. 3)), an arteriovenous (arteriolar/venular) hemoglobin oxygen saturation (sO_2) map (right column of each group (Fig. 3)), the blood volumetric flux rate (VFR), ear weight, and metabolic rate of oxygen (MRO₂) as measures of tumor metabolism (detailed in Methods).

Figure 2. *In vivo* microvascular imaging of a nude mouse ear. The maximum-amplitude-projection image was acquired *in vivo* by OR-PAM.

Figure 3. *In vivo* longitudinal OR-PAM imaging of tumor hemodynamics in a nude mouse ear. The left column is the maximum-amplitude-projection image, and color encoded for height. The right column is the sO₂ map simultaneously acquired. The vessel dilation was indicated by blue arrows.

Whole ear microvascular network imaging was longitudinally performed on days 7, 14, and 21 after tumor cell injection. The left column of Fig. 3 shows three-dimensional (3D) maps of microvascular imaging in the mouse ear. Vessel dilation was clearly identified at day 14 and 21, where trunk vessels after day 14 and 21 are larger than those on day 7 (indicated by blue arrows). The tumor shows progressive growth (Fig. 4a).

Moreover, the right column of Fig. 3 shows functional sO_2 imaging one days 7, 14, and 21. Interestingly, our measured sO_2 shows an increasing sO_2 value in the vein and tumor region. To provide a finer-grained view of oxygen distribution within the whole ear, we measured sO_2 in different veins within the whole ear (Fig. 4b). A functional PAM image of vein vessels on day 21 showed the highest sO_2 (0.82). These measurements uncovered an increasing sO_2 trend with time, but an opposite trend for OEF (Fig. 4c).

Next we examined whether the VFR was related to tumor growth. Remarkably, the VFR in the artery and vein were substantially elevated during tumor growth (6.2 ml/min d7; 8.1 ml/min d14; 18.9 ml/min d21). The rise in VFR was notable considering that the vasculature was remodeled after tumor injection. We reasoned that if arteriovenous (AV) shunting, or vessel adaption, happened, then vascular adaptation decreased the resistance of the microcirculation and to more blood flow. However, most of the blood flow drained to the vein via the short shunt pathway and therefore caused a low OEF. Moreover, increased VFR may possibly indicate that the tumor was starved for oxygen and nutrition.

Figure 4. *In vivo* longitudinal imaging of tumor hemodynamics in response to tumor growth in a nude mouse ear. (a) tumor volume, (b) sO_2 , (c) OEF, (d) volumetric flow rate, (e) MRO_2 .

3.3 *In vivo* longitudinal imaging revealed tumor oxygen hypermetabolism

These observations compelled us to investigate the MRO_2 in the mouse ear. Specifically, we asked whether the differences in the OEF and VFR could reveal the tumor growth mechanism. We calculated the MRO_2 from the OEF, VFR, and weight. Interestingly, the MRO_2 showed an increasing trend as the tumor grew (1.0 ml/100 g/min on d7; 1.1 ml/100 g/min on d14; 1.3 ml/100 g/min on d21). The OEF and VFR indicated that the vascular network responded to stimuli from tumor growth. The MRO_2 confirmed that the vessel carried more oxygen to the tumor tissue and the tumor tissue consumed more oxygen.

4. CONCLUSIONS

Direct measurements of absolute MRO_2 (demand) in renal tumor model have revealed a unique hyperdynamic landscape characterized its low OEF (structural adaptation) and high VFR (supply). The balance between supply and structural

adaptation can be altered by demand such as MRO_2 . ORPAM demonstrated its promise as a tool to noninvasively monitor hemodynamic parameters and to study oxygen metabolism in tumors.

5. ACKNOWLEDGMENTS

The authors appreciate the close reading of the manuscript by Prof. James Ballard. We also thank Junjie Yao for helpful discussions. This work was sponsored by National Institutes of Health Grants DP1 EB016986 (NIH Director's Pioneer Award), R01 CA186567 (NIH Director's Transformative Research Award), R01 CA159959, and R01 EB016963. L.V.W. has financial interests in Microphotoacoustics, Inc. and Endra, Inc., neither of which supported this work. K. Maslov has a financial interest in Microphotoacoustics, Inc.

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