Quantitative ultrasound molecular imaging for antiangiogenic therapy monitoring

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Abstract—The link between cancer growth and angiogenesis has led to the development of new techniques for cancer imaging and therapy. Ultrasound molecular imaging permits the visualization of angiogenesis by use of novel targeted ultrasound contrast agents, (tUCA), consisting of ligand-bearing microbubbles designed to specifically bind molecular angiogenic expressions. Discrimination between bound and free microbubbles is crucial to quantify angiogenesis. Currently, the degree of binding is assessed by the differential targeted enhancement, requiring the application of a destructive burst in the late phase (usually 5-10 min after injection) to isolate the signal from bound microbubbles. Recently, we proposed a novel method for quantitative assessment of binding by modeling the microbubble binding kinetics during the UCA first pass, reducing the acquisition time to 1 min with no need for a destructive burst. The feasibility of the method for angiogenesis imaging was shown in prostate tumor-bearing rats. In this work, we evaluate the proposed method for monitoring the response to angiogenic treatment in human colon cancer xenograft-bearing mice.

Index Terms—Angiogenesis imaging, antiangiogenic treatment, therapy monitoring, molecular imaging, pharmacokinetic modeling, cancer.

I. INTRODUCTION

Cancer is among the leading causes of death worldwide [1]. An established hallmark of cancer growth and development is angiogenesis, i.e., the process by which resting endothelial cells are recruited to form new blood vessels, needed to supply the tumor with oxygen and nutrients, and to provide it with an escape route for metastatic spread [2], [3], [4]. At a molecular level, this complex multistep process involves the activation of pro-angiogenic molecules, such as grow factors and integrins, and the interaction of various types of cells. At a vascular level, angiogenesis results in a chaotic vascular network of irregular, fragile, and tortuous types of cells. At a molecular level, this complex multistep process involves the activation of pro-angiogenic molecules, such as growth factors and integrins, and the interaction of various types of cells. At a vascular level, angiogenesis results in a chaotic vascular network of irregular, fragile, and tortuous micro-vascular exhibiting increased microvascular density and permeability [2], [3], [4].

Based on this, novel therapeutic strategies aim at inhibiting and/or disrupting angiogenic tumor vasculature by selectively targeting molecular expressions of cancer angiogenesis [5], [6]. Current approaches mainly include inhibition of tyrosine kinases pathways, whose aberrant activation is one of the most frequent mechanism of oncogenesis in humans, and blocking of the interaction with angiogenic ligands by use of monoclonal antibodies [5], [6]. An example of the latter is the anti-cancer drug bevacizumab (Avastin\textsuperscript{®}, Genentech/Roche), which specifically binds the vascular endothelial grow factor (VEGF), thereby blocking the interaction with its receptors. After FDA-approval in 2004 and EU-approval in 2005 [5], [7], bevacizumab has been used as first- and second-line of therapy for several cancer types, with proven improvements in patient survival [7].

The ongoing shift in the therapeutic strategy calls for a shift also in the evaluation methods. In fact, traditional survival-based and tumor size-based criteria are not suited for evaluating the early response to novel targeted anti-angiogenic and vascular disrupting therapies, in which alterations in size, if occurring, are a late event compared to functional changes [8], [9]. Moreover, early assessment is crucial for timely evaluation of new drugs, and for discrimination between responder and non-responder, which enables better tailoring of the therapeutic strategy [8]. As a result, molecular imaging of tumor angiogenesis holds great potential for cancer diagnosis and management, therapy monitoring, and drug development.

Low cost, wide availability, and absence of ionizing radiations make ultrasound molecular imaging (USMI) particularly suitable for in-vivo non-invasive assessment of cancer angiogenesis. In USMI, conventional ultrasound contrast agents (UCA), consisting of gas-filled microbubbles acting as echo-enhancers, are decorated with targeting ligands designed to bind selectively molecular expressions of angiogenesis [10]. In this context, the first clinically-translatable targeted UCA (tUCA) BR55 (Bracco Suisse, Geneva, Switzerland), targeting the VEGF receptor 2 (VEGFR2), has been recently developed and tested in a number of animal studies [10], [11], [12]. Currently, clinical trials in humans are ongoing to verify the feasibility of USMI with BR55 for detection of prostate, breast, and ovarian cancer [10]. After intravenous injection of a bolus of BR55, microbubbles can flow through the entire circulation and attach specifically to cells expressing VEGFR2, thus causing selective enhancement in areas of active angiogenesis, especially in the late phase, several minutes after injection.

Although the lack of proportionality between the acoustic signal and the absolute level of biomarker expression hampers quantification, discrimination of the signal coming from free and bound microbubbles may provide an indirect way of quantifying of angiogenesis. Conventionally, this is done by applying a destructive ultrasound burst at high-MI to destroy all the microbubbles present in the acoustic field, and observing the replenishment by the freely flowing microbubbles.
Calculation of the difference in the acoustic intensity before and after the application of the high-MI burst (differential targeted enhancement, dTE) enables isolating the signal coming from bound microbubbles only [13], [14]. Although simple and rather straightforward to implement, this method lacks reproducibility, due to the arbitrary choice of the time points used for analysis, and it is not quantitative, due to the dependency on attenuation and machine settings. Moreover, the method requires lengthy procedures, which can go up to 30 minutes, and the use of a destructive high-MI pulse raises concerns for damages to the endothelial tissue [15]. These limitations particularly hamper therapy monitoring, whereby several repeated measurements are compared.

In response, we recently proposed a novel method for quantitative USMI based on mathematical modeling of the tUCA binding kinetics in the first-pass, requiring shorter acquisition time and no need for the application of a destructive burst [16]. After showing its feasibility for angiogenesis imaging in prostate tumor-bearing rats, in this work, we test the proposed method for monitoring therapy response to anti-angiogenic treatment in colon cancer xenograft-bearing mice treated with bevacizumab.

II. METHODS

A. Modeling of microbubble binding kinetics

The total concentration, \(C_t(t)\), of a tUCA in a pixel of tissue is described by the weighted sum of the concentrations of free microbubbles, \(C_f(t)\), and bound microbubbles, \(C_b(t)\). By modeling the transport of free microbubbles as a convective-dispersion process, \(C_f(t)\) can be described by the local density random walk model [17]; while, with the assumption of negligible unbinding in the first-pass of the tUCA bolus, the kinetics of bound microbubbles can be described as an accumulating well-mixed compartment. The resulting first-pass binding (FPB) model is given below [16]

\[
C_t(t) = v_f C_f(t) + K_b \ast C_f(t) = \\
v_f \alpha \sqrt{\frac{\kappa}{2\pi}} (t - t_0)^{-1/2} e^{-\frac{(t - t_0)^2}{2(t - t_0)}} + \\
K_b \alpha \sqrt{\frac{\kappa}{2\pi}} \int_0^{t-f\text{lash}} (\tau - t_0)^{-1/2} e^{-\frac{\kappa(\tau-t_0)^2}{2(\tau-t_0)}} d\tau
\]

where \(v_f\) is the fractional dilution volume of free microbubbles; \(K_b\) is the microbubble binding rate; \(\alpha\) is the time-integral of \(C_f(t)\); \(t_0\) is the theoretical contrast injection time; \(\mu\) is the mean transit time of the free microbubbles between injection and detection sites; and \(\kappa\) is the dispersion parameter, given by the local ratio between contrast convection (squared velocity \(v^2\)) and dispersion (dispersion coefficient \(D\)).

B. Tumor model in mice

Colon cancer xenograft tumors were established in Charles River female mice. Clinical responders were simulated by injection of human colon cancer cell lines LS174T (ATCC, Manassas, VA, USA), which has shown resensitivty to VEGF-targeted therapy [19]. Both LS174T (n=2) and CT26 (n=1) tumors were allowed to grow 10 days before treatment. At days 0/1, 3, and 7, one LS174T tumor-bearing mouse (responder) and one CT26 bearing mouse (non-responder) received bevacizumab (Avastin, 10-mg/kg intravenous injection; Genentech, San Francisco, CA, USA), while the remaining mouse was injected with saline (control).

The study was approved by the Institutional Administrative Panel on Laboratory Animal Care of Stanford University.

C. Ultrasound molecular imaging

Three dimensional USMI was performed at days 0/1, 1/2, 3, 7, 10 using an Epiq 7 ultrasound scanner (Philips Healthcare, Andover, MA, USA) with an X6-1 matrix array transducer working at 3.2 MHz in power modulation contrast imaging mode (mechanical index, MI = 0.09; volume sampling rate = 1 Hz; dynamic range = 52 dB; focal depth = 5 cm). A bolus of BR55 (5x10^7 microbubbles, 100 \(\mu\)L; Bracco Suisse, Geneva, Switzerland) was injected in the mice tail vein using an infusion pump (Kent Scientific, Torrington, CT, USA). About four minutes after injection \(t = t_{\text{flash}}\) a destructive US pulse was applied by switching the MI to 0.72 for a duration of 2 seconds. After the high-MI pulse, imaging at low MI was continued for 2-3 minutes.

D. Ultrasound quantification

For each 2D plane of the 3D USMI dataset, time intensity curves (TICs) were extracted at each pixel and fitted by the FPB model in (1). To exclude recirculation, only the first 55 s of the curve were analyzed. All the analysis was implemented in MATLAB®/Natick, MA, USA) as explained in [16]. The binding rate \(K_b\) was estimated at each pixel and compared with the late-enhancement (signal intensity [SI] for \(t > t_{\text{flash}}\) - 40s) and dTE (average SI for \(t_{\text{flash}} - 130s < t < t_{\text{flash}} - 30s\) subtracted by the average SI for \(t > t_{\text{flash}} + 30s\)).

To evaluate the ability of the binding parameter \(K_b\) to monitor the response to anti-angiogenic therapy, regions-of-interest (ROIs) around the cancer area were drawn in the three most central US planes. The average \(K_b\), dTE, and late-enhancement were calculated at days 0/1, 1/2, 3, 7, and 10, and the difference between the average parameter values at days 0/1 and 10 was tested by a two-sample Student t-test with level of significance \(\alpha = 0.01\).

E. Histological quantification

After extraction, fixation in paraformaldehyde, and cryopreservation in a sucrose solution, tumors were frozen and sectioned into 1-mm slices. Out of each slice, a 10-\(\mu\)m section was obtained from the center and used for quantification of VEGFR2 expression and percentage area blood vessels, by standard immunofluorescence and CD31 staining, respectively. Multiple confocal microscopic images were obtained and both VEGFR2 expression and percentage area blood vessels were quantified as the average value from at least 5 randomly selected fields of view in the different tumor slices. Finally, the overall mean values per tumor volume were calculating by averaging the values over all the slices.
Fig. 1. Binding rate $K_b$ (blue), dTE (red), and late enhancement (green) evaluated at five time points in one responder, one non-responder (treated with bevacizumab), and one control (treated with saline). Error bars indicate the standard deviation.

III. RESULTS

The changes in the mean $K_b$, dTE, and late-enhancement values during treatment are shown in Fig. 1. The differences in the parameters values pre-treatment (day 0/1) and post-treatment (day 10) are shown in Fig. 2 and evaluated in Table I (two-sample Student t-test). Table II compares the values of the parameters post-treatment (day 10) with the histological quantification of the tumor after resection, evaluated in terms of VEGFR2 expression levels and percentage of blood-vessel area.

IV. DISCUSSION AND CONCLUSIONS

The development of novel anti-angiogenic cancer therapies evidences a need for imaging methods for in-vivo quantitative assessment of cancer angiogenesis. Recently, we proposed quantitative USMI by modeling the binding kinetics of tUCAs with the FPB model. Fitting TICs obtained at each imaging pixel by this model enables quantitative assessment of angiogenesis by the estimation of the binding rate $K_b$. In this work, the feasibility of the proposed method in the context of anti-angiogenic therapy monitoring has been tested in colon cancer xenograft-bearing mice treated with bevacizumab.

The proposed binding parameter $K_b$ decreased significantly in the responder mouse, while no significant differences were
observed in the non-responder and in the control (Table I). Moreover, higher $K_b$ values after treatment (day 10) were found in the control and the non-responder compared to the responder, which was in agreement with the histological quantification of extracted tumors, evaluated in terms of expression of VEGFR2 and percentage blood vessel area (Table II).

Compared to conventional US quantification methods, although the late-enhancement and the dTE showed greater separation between the pre- and post-treatment values in the responder (Fig. 2), they also showed significant differences in the non-responder (late-enhancement) and in the control (both) (Table I), suggesting lower reproducibility.

To conclude, the proposed binding parameter $K_b$ showed to be a suitable quantitative biomarker for monitoring the therapeutic response to anti-VEGF treatment, potentially overcoming some of the limitations of current techniques, including low reproducibility, long acquisition times, and the need for an high-pressure US pulse (dTE). However, until now the investigation was limited to three mice only. Therefore, further pre-clinical and clinical validation are needed to confirm the promising role of the binding parameter $K_b$ for anti-angiogenic therapy monitoring, and potentially pave the way towards the clinical translation of quantitative USMI.

Acknowledgment

This work was supported by the European Research Council (ERC), Starting Grant 280209, by the NIH R01 CA155289-01 grant (JKW) and R01DK092509-01 grant (JKW).

References