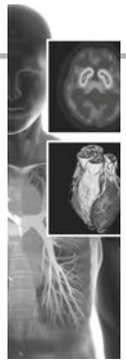

Imaging tortuosity: the potential utility of acoustic angiography in cancer detection and tumor assessment

"...tumor-associated vascular abnormalities were detectable and quantifiable using a combination of a high-resolution contrast-enhanced ultrasound imaging approach ('acoustic angiography') and postprocessing analysis..."

KEYWORDS: angiogenesis ■ contrast ■ microbubbles ■ microvasculature ■ tortuosity ■ ultrasound

The morphology and structure of the vasculature associated with malignant tumors has long been observed to be chaotic and unusual compared with that of healthy tissues. In tumors, vessel diameters and branching patterns appear random, and the actual trajectories charted through the 3D tumor volume are often tortuous [1,2]. These morphological abnormalities are hypothesized to be a result of a number of complicated physiological effects occurring in parallel, such as tumor cells' high metabolic demand and their unusual microenvironment (hypoxic, acidic and so on). These factors contribute to a heightened level of angiogenic activity in and around the tumor, which becomes a self-amplifying cycle when the newly formed vessels fail to supply the tumor cells' insatiable appetite for nutrients [3]. The heightened angiogenic activity also causes the basement membrane and support structure of the vessels to break down. Additionally, the poor organization of the vessel network results in an increase in pressure within the vessels, which is further exacerbated by the absence of lymphatic drainage from the tumor stroma.

Of interest from a cancer screening perspective, tumor-associated vascular irregularities can extend beyond the margins of a lesion, as angiogenic growth factors promoted by tumor cells can influence the morphologies of even major vessels in the vicinity of tumors [4]. Additionally, cancer-associated tortuous vessel morphologies have been observed in animal models much sooner than the arrival of a palpable mass. For example, vascular remodeling has been observed via microscopy and window chamber models when only tens to hundreds of tumor cells are present within an otherwise healthy tissue volume [5].

The renormalization of tumor vasculature is also of great interest to researchers and clinicians alike, as anticancer therapies, when effectively quelling a tumor's progression, have been shown to have a rapid effect on the local vascular morphology. This normalization of vessels has served as an indication of eventual tumor volume reduction and the lack of normalization has correspondingly been associated with poor therapeutic response [6].

Currently, it is challenging to identify the presence of abnormal vasculature associated with tumor presence, as the aberrant features (vessel tortuosity, branching patterns and so on) are not readily detected with most clinically utilized modalities. Recently, however, our research team arrived at an encouraging result. Using a rodent model, we demonstrated that tumor-associated vascular abnormalities were detectable and quantifiable using a combination of a high-resolution contrast-enhanced ultrasound imaging approach ('acoustic angiography') and postprocessing analysis of the vessel morphologies [7]. We have dubbed this combination of imaging and vessel analysis 'microvascular-mapping'. Although our method is not the first example of such analysis [8–10], our approach involves higher resolution and greater signal-to-noise contrast imaging than described in previous studies. As ultrasound is an innocuous, relatively inexpensive and widely available imaging modality, such strategies capable of detecting vessel abnormalities associated with the arrival or presence of cancer, or capable of assessing tumor response to therapy, could be powerful tools in clinical cancer diagnostics.

How then can one detect and quantify aberrant vasculature in either preclinical or clinical images, and use this in a diagnostic context? The

Ryan C Gessner

Joint Department of Biomedical Engineering, The University of North Carolina & North Carolina State University, University of North Carolina Campus Box 7575, Chapel Hill, NC 27599, USA

Stephen R Aylward

Department of Medical Imaging, Kitware, Carroboro, NC 27510, USA

Paul A Dayton

Author for correspondence: Joint Department of Biomedical Engineering, The University of North Carolina & North Carolina State University, University of North Carolina Campus Box 7575, Chapel Hill, NC 27599, USA
Tel.: +1 919 843 9521
padayton@bme.unc.edu

Future Medicine part of fsg

morphology and makeup of tumor vasculature diverge from healthy vasculature in several ways and these differences can be elucidated by either direct or indirect observations. For direct observations (i.e., quantification of 3D vessel morphologies) there are three critical components. First, the imaging modality must have a high enough spatial resolution to enable individual vessels to be resolved and it must have sufficient signal-to-noise to enable differentiation of vessels from surrounding tissue. Second, a segmentation approach must be able to extract vessels from the image volumes in a repeatable and reliable way. This stage usually requires some user input and feedback, although automated approaches allow for higher throughput and thus would be more amenable to clinical implementation. Finally, a classification algorithm based on the segmented vessel data that indicates the presence or degree of pathology is needed. Morphological features can be examined at either the vessel level (e.g., the tortuosity of a single vessel) or at the network level (e.g., the fractal dimension of the vessel tree). Indirect observations of cancer-associated vascular abnormalities are far more prevalent in the clinic today. Examples include measures of overall tissue perfusion and vessel permeability. These types of measurements are powerful indications of tumor presence and malignancy, but they are limited to more global estimations of vessel morphology. These two types of observations (direct and indirect) have been shown to provide independently useful diagnostic information [11]. It is, therefore, unlikely that direct assessments of vascular structure will ever replace existing cancer imaging approaches; however, rather they will compliment them with additional diagnostically powerful information. This could result in a hybrid cancer-assessment approach that is more sensitive than either component alone.

> *"Currently, it is challenging to identify the presence of abnormal vasculature associated with tumor presence, as the aberrant features ... are not readily detected with most clinically utilized modalities."*

Given the success of previous work related to quantifying vascular morphology for cancer assessment, the promising imaging quality achieved and the quantitative results demonstrated to date, we believe that acoustic angiography and microvascular mapping could have a large impact in preclinical and clinical cancer diagnostics. In the clinic, it could enable a bedside tool for rapid image acquisition, resulting in useful quantitative metrics to assist clinicians in fine-tuning patient-specific treatment plans. Owing to the high frequencies used to create high-resolution images with the acoustic angiography approach, only superficial cancers could be imaged, such as thyroid, melanoma, and breast lesions. However, with modifications to transducer-form factor (such as transrectal- or catheter-based designs), the approach could also be implemented in prostate imaging or atherosclerotic plaque assessment. Preclinical researchers are not faced with the same depth restrictions, as their mouse and rat models are comparatively small, and thus acoustic angiography-based microvascular mapping would enable candidate cancer therapeutics to be efficiently and noninvasively assessed in a wider array of tumor types.

> *"...we believe that acoustic angiography and microvascular mapping could have a large impact in preclinical and clinical cancer diagnostics."*

Limitations to the acoustic angiography and vessel mapping approach are severalfold. Currently, acoustic angiography with high resolution and sufficiently low background signal for reliable vessel segmentation requires transducer technology that is still in the research and development stage and not yet commercially available [12]. Furthermore, this technique requires the use of ultrasound contrast agents, which, despite their potential, have been slow to achieve acceptance for noncardiac applications, particularly in the USA. Finally, image segmentation and classification techniques to characterize vessel morphologies will require further development and training to achieve the level of automation, sensitivity and specificity required for a clinical tool.

Despite these challenges, the potential of using ultrasound to image microvessels as a 'fingerprint' of cancer, adjunct to the tumor mass itself, is an exciting prospect, which may provide a powerful new avenue for describing and classifying solid tumors.

Financial & competing interests disclosure

P Dayton is on the Scientific Advisory Board for Targeson, Inc. Funding for related research was provided by the NIH, grants R01-CA138419, R01-EB009066, R01-CA170665 and R43-CA165621, as well as pilot study funds from the University of North Carolina Lineberger Comprehensive Cancer Center. A pilot study grant from Lantheus Medical

Imaging provided Definity contrast agent, which was used in some preliminary studies. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

1 Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. *Science* 307(5706), 58–62 (2005).

2 Fukumura D, Duda DG, Munn LL, Jain RK. Tumor microvasculature and microenvironment: novel insights through intravital imaging in pre-clinical models. *Microcirculation* 17(3), 206–225 (2010).

3 Carmeliet P, Jain RK. Principles and mechanisms of vessel normalization for cancer and other angiogenic diseases. *Nat. Rev. Drug Discov.* 10(6), 417–427 (2011).

4 Bullitt E, Lin NU, Smith JK *et al.* Blood vessel morphologic changes as visualized by MR angiography during treatment of brain metastases: a feasibility study. *Radiology* 245(3), 824–830 (2007).

5 Li CY, Shan S, Huang Q *et al.* Initial stages of tumor cell-induced angiogenesis: evaluation via skin window chambers in rodent models. *J. Natl Cancer Inst.* 92(2), 143–147 (2000).

6 Bullitt E, Ewend M, Vredenburgh J *et al.* Computerized assessment of vessel morphological changes during treatment of glioblastoma multiforme: report of a case imaged serially by MRA over four years. *Neuroimage* 47(Suppl. 2), T143–T151 (2009).

7 Gessner RC, Aylward SR, Dayton PA. Mapping microvasculature with acoustic angiography yields quantifiable differences between healthy and tumor-bearing tissue volumes in a rodent model. *Radiology* 264(3), 733–740 (2012).

8 Molinari F, Mantovani A, Deandrea M, Limone P, Garberoglio R, Suri JS. Characterization of single thyroid nodules by contrast-enhanced 3-D ultrasound. *Ultrasound Med. Biol.* 36(10), 1616–1625 (2010).

9 Huang SF, Chang RF, Moon WK, Lee YH, Chen DR, Suri JS. Analysis of tumor vascularity using three-dimensional power Doppler ultrasound images. *IEEE Trans. Med. Imaging* 27(3), 320–330 (2008).

10 Chang YC, Huang YH, Huang CS, Chang RF. Vascular morphology and tortuosity analysis of breast tumor inside and outside contour by 3-D power Doppler ultrasound. *Ultrasound Med. Biol.* 38(11), 1859–1869 (2012).

11 Parikh AH, Smith JK, Ewend MG, Bullitt E. Correlation of MR perfusion imaging and vessel tortuosity parameters in assessment of intracranial neoplasms. *Technol. Cancer Res. Treat.* 3(6), 585–590 (2004).

12 Gessner R, Lukacs M, Lee M, Cherin E, Foster FS, Dayton PA. High-resolution, high-contrast ultrasound imaging using a prototype dual-frequency transducer: *in vitro* and *in vivo* studies. *IEEE Trans. Ultrason. Ferroelectr. Freq. Control* 57(8), 1772–1781 (2010).