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● *Original Contribution*

ELASTOGRAPHIC ASSESSMENT OF XENOGRAFT PANCREATIC TUMORS

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Abstract—High tissue pressures prevent chemotherapeutics from reaching the parenchyma of pancreatic ductal adenocarcinoma, which makes it difficult to treat this aggressive disease. Researchers currently use invasive probes to monitor the effectiveness of pressure-reducing therapies, but this practice introduces additional complications. Here, we hypothesize that Young's modulus is a good surrogate for tissue pressure because collagen density and hyaluoronic acid, the key features of the tumor microenvironment responsible for high tissue pressures, also affect modulus elastograms. To corroborate this hypothesis, we used model-based quasi-static elastography to assess how the Young's modulus of naturally occurring AsPc-1 pancreatic tumors varies with collagen density and hyaluoronic acid concentration. We observed that Young's moduli of orthotopically grown xenograft tumors were 6 kPa ($p < 0.05$) higher than that of their subcutaneously grown counterparts. We also observed a strong correlation between Young's modulus and regions within the tumors with high collagen ($R^2 \approx 0.8$) and hyaluoronic acid ($R^2 \approx 0.6$) densities. These preliminary results indicate that hyaluronic acid and collagen density, features of the pancreatic ductal adenocarcinoma tumor microenvironment responsible for high tissue pressure, influence Young's modulus. (E-mail: m.doyley@rochester.edu

Key Words: **Tumor microenvironment, Model-based elastography, Pancreatic ductal adenocarcinoma, Total tissue pressure.**

INTRODUCTION

Several factors contribute to the poor prognosis of pancreatic cancer, but late diagnosis is the most significant (Fass 2008; Miles 1999). Pancreatic ductal adenocarcinoma (PDA) has a 5-year survival rate of less than 6% (Gore and Korc 2014). The advanced stage of the disease, often metastasized to distant organs when first diagnosed, is responsible for this dismal prognosis. Contrast-based imaging techniques such as magnetic resonance imaging and X-ray computed tomography can visualize structured tumors (Fass 2008), but PDA is avascular (Miles 1999), which reduces the delivery of contrast agent to the tumor, thus degrading diagnostic efficacy.

Elastography (Doyley and Parker 2014; Maleke and Konofagou 2008; McAleavey et al. 2007; Nightingale et al. 2002; Urban et al. 2006) can improve the differential diagnosis of pancreatic tumors and lymph nodes. Clinicians routinely use endoscopic ultrasound (EUS) to guide fine-needle aspiration and biopsy (Chantarojanasiri et al. 2016; Wangermez 2016). However, EUS-guided biopsy is difficult, often requiring multiple punctures to obtain a sufficient number of tissue samples. Several researchers have reported that endoscopic ultrasound elastography can differentiate benign from malignant pancreatic tumors and lymph nodes with high accuracy (Cui et al. 2015; Iglesias-Garcia et al. 2017). Despite these encouraging results, endoscopic ultrasound elastography is invasive and currently only available on one commercially available system, Hitachi's EUB-8500 system. To overcome these issues, Chen et al. (2015) determined that non-invasive elastographic techniques like harmonic motion imaging could also improve the differential diagnosis of pancreatic cancer.

Pancreatic ductal adenocarcinoma is hard to eradicate because high tissue pressures prevent chemotherapeutics from reaching the tumor parenchyma (Boucher et al. 1991, 1997; Less et al. 1992; Roh et al. 1991). Radical surgical resection is the current cure for PDA, but only 15% to 20% of patients have resectable disease (Yendluri et al. 2007). Neo-adjuvant therapies can help patients with

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borderline resectable tumors qualify for surgery, but high tissue pressure (Chu et al. 2007; Jain 1998, 2011; Vakoc et al. 2009) impedes drug delivery, which produces hypoxia that encourages tumor progression and reduces the efficacy of radiotherapy and chemotherapy. To solve this problem, researchers have developed targeted therapies to degrade either stromal density (Olive et al. 2009) or hyaluronic acid (Cowell et al. 2015; Dychter et al. 2011; Hingorani et al. 2015), features of the tumor microenvironment responsible for high tissue pressures (Chauhan et al. 2014; Provenzano et al. 2012).

In this study, we hypothesized that model-based quasi-static elastography can provide a good surrogate for tissue pressure. No imaging modality can measure tissue pressure directly. Consequently, clinical researchers frequently use probes to measure tissue pressure (Boucher et al. 1997; Griffon-Etienne et al. 1997; Gutmann et al. 1992; Jain and Baxter 1988; Stylianopoulos et al. 2012). Specifically, researchers have used probes to assess how tissue pressure affects patient survival (Curti et al. 1993). However, pressure probes are invasive, which introduces additional complications and errors. The pressure gradient at PDA tumor margins (Jain 1987) increases Young's modulus (Swartz and Lund 2012). Consequently, we hypothesized that Young's modulus measured with quantitative elastographic imaging methods, such as model-based quasi-static elastography and shear wave imaging, is a good surrogate for tissue pressure. To confirm this hypothesis, we performed studies on human-derived AsPc-1 tumors, among the hardest to treat clinically. Specifically, we conducted studies on immunocompromised mice and rats to assess (i) the correlation between tissue stiffness and interstitial pressure, (ii) whether there is a significant difference in Young's modulus between orthotopically and subcutaneously grown xenograft tumors and (iii) how tissue stiffness varies with stromal density and hyaluronic acid content, features of the tumor microenvironment responsible for high tissue pressures.

METHODS

In this section, we describe the tumor model, pressure measurement procedure, elastographic imaging protocol, histological analysis and statistical analysis performed on the acquired data.

Tumor model

We conducted experiments on AsPc-1 xenograft tumors. We grew tumors by injecting 1×10^6 tumor cells in Matrigel (BD Biosciences, San Jose, CA, USA), and 50% media, either subcutaneously into the right flank or orthotopically into the pancreas. We allowed all tumors to grow until they reached 125–175 mm^3 in size. All animal studies were performed using protocols approved by the institutional animal care and use committees of the University of Rochester and Dartmouth College.

Pressure measurements

We used a Mikro-Tip piezo-electric pressure catheter (Model SPR-671, Millar, Houston, TX, USA; 0.47-mm diameter, dynamic pressure range from −50 to 300 mm Hg and nominal sensitivity of 5 μV/mm Hg) to measure the total pressure within the tumors. A LabPro data acquisition unit (Vernier Software and Technology, Beaverton, OR, USA) digitized all pressure data to 8 bits at a sampling rate of 60 samples per minute.

Histological analysis

To facilitate quasi-static elastographic imaging, we removed the tumors from the animals and embedded them in gelatin (see the next subsection). After imaging, we removed the excised tumors from the gelatin block and snap-froze them for later Masson trichrome and hyaluronan (Jacobetz et al. 2013) staining. All samples were sectioned into 5-μm-thick slices, taken at 100-μm intervals. The stained tissues were digitally captured with a Vectra 3 slide scanner (Perkin Elmer, Waltham, MA, USA). We used a two-step process to generate collagen and hyaluronic acid maps. First, we transformed the digitized histological images from red, green and blue (RGB) to hue, saturation and value (HSV) color space. Second, we used a global thresholding algorithm to segment the transformed images: blue for collagen and brown for hyaluronic acid. We performed all quantitative histological analyses in a MATLAB (The MathWorks, Natick, MA, USA) programming environment.

Elastographic imaging

Tumor encasement. All tumors were surgically removed and encased in a $57 \times 25 \times 42$-mm (width × height × thickness) gelatin block as described in Doyley et al. (1999). We manufactured the gelatin block from a suspension consisting of 15% by weight porcine skin gelatin (300 bloom, Type A, Sigma-Aldrich, St. Louis, MO, USA), 2% by weight corn-starch (Spectrum Pharmaceuticals, Henderson, NV, USA) and 18 MΩ high-purity water.

Data acquisition. We used the experimental setup illustrated in Figure 1 to acquire elastographic images. This system consisted of a SonixTouch ultrasound scanner (BK Ultrasound, Peabody, MA, USA), a L40-8/12 probe (BK Ultrasound) and a computer-controlled mechanical compression system. All echo imaging was performed at 10 MHz; the resulting radiofrequency (RF) echo data were digitized to 10 bits at a sampling rate of 40 MHz. During elastographic imaging, the encapsulated samples were deformed at a strain rate of 2%/s. For each sample, we