Tumor Stiffness Dependency on Tissue Viability as Measured Using MR Elastography (MRE)

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Introduction: Previous studies indicate that MRE-derived tumor stiffness holds promise as a biomarker of response to chemotherapy [1]. We propose that this measure can also be applied to quantify other biologically significant events including degree of treatment response and tumor viability. The purpose of this study was to quantify MRE-derived stiffness in a mouse tumor model under conditions of suboptimal chemotherapy dosage as well as viability of the host. We hypothesize that in vivo tumor stiffness measures can quantify *both* sub-lethal dose response and overall tumor viability. The target audience of this work is MR scientists and clinicians involved in developing techniques for actively monitoring therapeutic response.

Methods and Materials: Tumors were grown in 20 mice in accordance with our institutional animal care and use committee (IACUC) approved protocol. DoHH2 (non-Hodgkin's lymphoma) cells were injected subcutaneously and grown for 6-8 weeks (one mouse failed to grow a tumor and was excluded) [2]. Following an initial scan to determine baseline stiffness, all mice received an intraperitoneal injection of 4mg of cyclophosphamide (160mg/kg). Each mouse was scanned 4 days post treatment to observe a change in stiffness following chemotherapy [1]. Mice were subsequently divided into two independent groups to assess changes in tumor stiffness following expiration of the host (n = 10) as well as the longitudinal effect of administration of a sub-lethal dose of chemotherapy (n = 9). The first group was sacrificed using an inhalation of CO_2 and scanned within 1 hour post mortem. The second group was monitored until an observable decrease in tumor size was detected. The mice were subsequently scanned on day 18 (n = 4), day 28 (n = 5) and day 35 (n = 2). Visible signs of tumor growth were observed at each time point. MRE was performed on each mouse using the experimental setup shown in Figure 1. A frequency of 800Hz was chosen to optimize localized vibrations with minimal shear wave attenuation throughout the tumor. All experiments were performed on a 3.0T whole-body MR scanner (GE Healthcare, Waukesha WI, USA) using a custom-build, 6-cm diameter, transmit-receive RF coil. A SE-EPI MRE sequence was used with the following imaging parameters: FOV = 5 cm, coronal image plane, TR/TE = 1100.0/99.3 ms, 4 contiguous slices, 2-mm slice thickness, motion-encoding gradient (MEG) frequency = 800Hz, 60 MEG pairs, through-plane MEG direction, 3 phase offsets, motion-encoding sensitivity = 4.0 μm/(π radians), and BW = ±41.5 kHz. During imaging, the mice were kept under general anesthesia using a steady flow of isoflurane and were allowed to breathe freely. Tissue stiffness maps (elastograms) were calculated using a Multiple Model Direct Inversion (MMDI) algorithm with 2D directional filters (radial Butterworth band pass filter cutoff frequencies of 2 and 128 cycles/FOV) [3-4]. A caliper (GENERAL, 6" Dial Caliper) was used to obtain volume measurements as an indication of response.

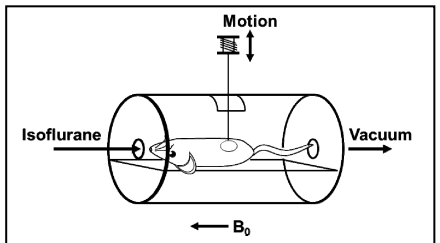

Figure 1: Experimental setup. A fine, MRI-compatible needle was inserted into the tumor and attached to a high-frequency electromechanical driver.

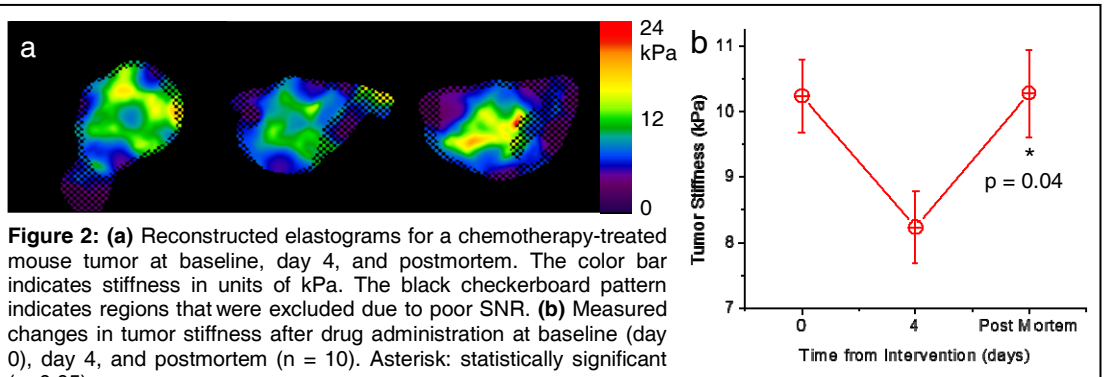

Figure 2: (a) Reconstructed elastograms for a chemotherapy-treated mouse tumor at baseline, day 4, and postmortem. The color bar indicates stiffness in units of kPa. The black checkerboard pattern indicates regions that were excluded due to poor SNR. **(b)** Measured changes in tumor stiffness after drug administration at baseline (day 0), day 4, and postmortem (n = 10). Asterisk: statistically significant ($p<0.05$).

Results: The reconstructed elastograms for a single mouse at baseline, 4 days following treatment and 1 hour post-mortem are shown in Figure 2a. The average measured stiffness of all 10 mice at each time point is shown in Figure 2b. At day 4, the stiffness decreased from baseline (p = 0.06, paired student's t-test, n = 10) and then significantly increased postmortem (p = 0.04, paired student's t-test, n = 10). Figure 3 is a plot of average tumor stiffness and volume versus time following chemotherapy administration. Both parameters show evidence of an initial, sub-lethal response followed by subsequent tumor recovery and growth.

Conclusions: These data confirm the hypothesis that *both* response to therapy and viability of the tumor, as determined by survival of the host, can be quantified by MRE. In addition, these data highlight the significance of physiological effects such as tumor perfusion and diffusion by demonstrating a large change in stiffness following host expiration prior to rigor mortis. In contrast to ex vivo studies which have demonstrated that chemotherapy results in an increase in stiffness of both the tumor and individual malignant cells [5,6], MRE-derived tumor stiffness demonstrated a decrease in stiffness within 4 days post treatment. These data highlight the need for biomarkers that can assess therapeutic response under noninvasive, in vivo conditions. MRE is well suited to noninvasively determine tissue shear stiffness in many regions throughout the body. Therefore, MRE has strong potential as a biomarker of changes in tissue mechanical properties that occur in response to chemotherapy. Future efforts will involve additional animal models, including a control group and extended time points post mortem.

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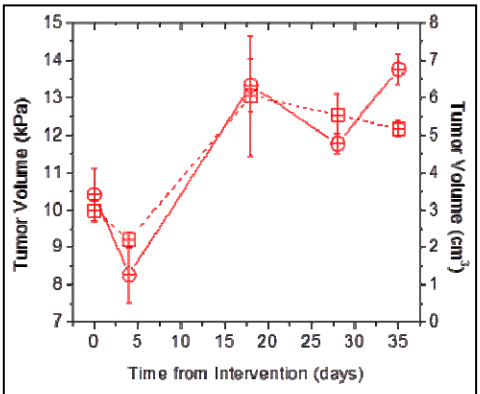

Figure 3: Change in tumor stiffness (solid line) and volume (dashed line) with time (n = 9). Chemotherapy treatment occurred on day 0.

References:

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