Non-invasive Imaging and Quantitative Readout of HCC Progression and Metastatic Development Using MRI

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Purpose:

Hepatocellular Carcinoma (HCC), the most common form of liver cancer, is the 3rd leading cause of cancer deaths worldwide[1]. Current treatments include surgery, liver transplant, and chemotherapy. New treatments that are less invasive with minimal toxicity are urgently needed. In this study, we propose a novel method of in-vivo MRI to monitor HCC progression in Huh7 mice, using two types of image contrast for effective HCC identification, as well as tumor volume readout. These methods were applied for randomization to treatment (confidential) or placebo, in order to verify on-target effects and treatment efficacy.

Methods:

A total of 15 mice (female, SCID-beige, 10 weeks) were imaged weekly starting at 3 weeks after orthotopic Huh7 cell implantation (IACUC protocol 09NBC083). During imaging, animals were maintained under anesthesia using 2.0% Isoflurane delivered in oxygen at 1.5L/min. Body temperature and respiratory rate were monitored and maintained at 37C and 40 RPM using SAII Small Animal Monitoring System (SA Instruments Inc., New York, NY). MR images were acquired using a 4.7T Bruker Pharmascan (Bruker Biospin, Billerica, MA) with a 38mm volume resonator. MDEFT (Modified Driven Equilibrium Fourier Transform) was used to get negative contrast for tumor, with TR=2000ms, TE=4.6ms, TI=900ms, 8 averages and scan time=17min4s. RARE (Rapid Acquisition Relaxation Enhancement) was used to get positive contrast for tumor, with TR=2200ms, TE=42.9ms, 20 Averages and Scan Time=18min20s. Both methods used the same geometry definition, with Field-of-View (FOV)=40×40mm, Matrix=256×256, 16 coronal slices, and Slice Thickness=0.55mm. Anatomical coverage extended from the top of the chest cavity to the bottoms of kidneys.

Data Analysis:

HCC volume measurement was done using Amira V5.3 software (Visualization Science Group, Burlington, MA). Tumors were segmented from the RARE images with MDEFT images used for boundary verification. Contrast to Noise (CNR) values were calculated for tumor and intestine versus healthy liver.
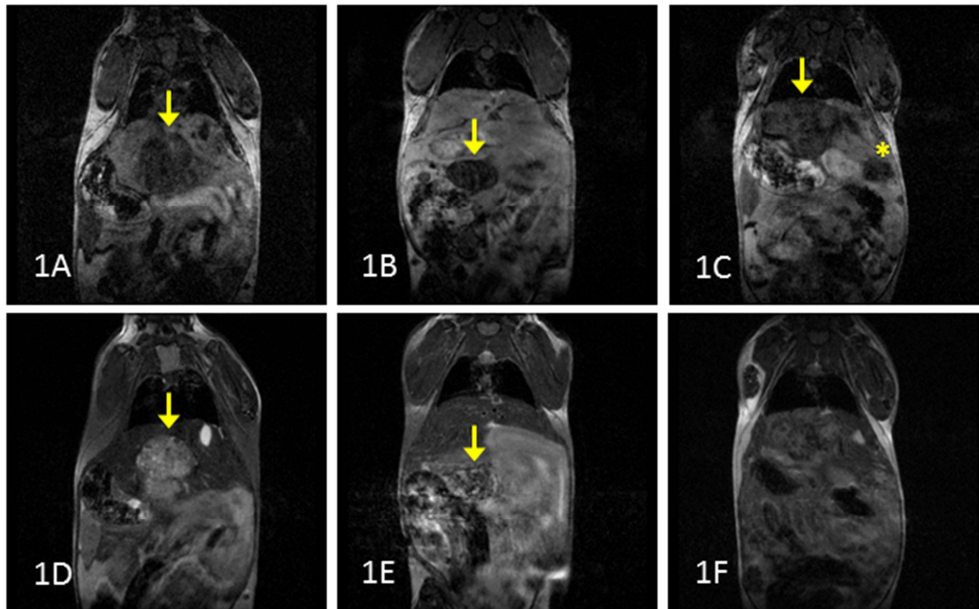

Figure 1. Combined MDEFT/RARE for three representative HCCs. Top row: MDEFT. Bottom row: RARE.

Results:

Figure 1 shows representative images from Huh7 mice. HCC #1 (Fig 1A,1D; arrow) has high CNR on MDEFT (CNR=9.5) and RARE (CNR=38.5), with clear identification of tumor and tumor margins. HCC #2 (Fig 1B,1E; arrow) has high CNR on MDEFT (CNR=29.5) and RARE (CNR=18.5), however, given the proximity to the intestines, identification of tumor margins is only possible on MDEFT. HCC #3 (Fig 1C,1F, arrow) shows the versatility of MDEFT in identifying primary HCC (arrow) and secondary liver metastasis (asterisk) adjacent to the stomach and intestines. CNR was 24.2 for MDEFT versus 4.6 for RARE (comparable to intestine CNR=6.9). At 3 weeks, 4 weeks, and 5 weeks after orthotopic Huh7 cell implantation, mice were randomized to treatment (confidential) or placebo. Tumor volumes ranged from 15 – 1205 mm^3, with a median volume of 246 mm^3. Greater than 80% knockdown of target mRNA expression was observed, indicated positive on-target effects. The second phase of this study is currently ongoing, with MRI serving as the primary readout for randomization to treatment, and treatment efficacy. Concomitant serum biomarker development is underway for translation to the clinic.

Discussion/Conclusions:

In this study, we were able to diagnose and quantify primary HCC tumor volume and secondary liver metastasis *without* contrast agent and *without* respiratory triggering. We achieved higher CNR than previous reports employing Gd-DTPA[1] or Gd-EOB-DTPA[1-2], with a detection limit of 1mm diameter HCC versus 2mm reported[3]. In summary, combined MDEFT/RARE allows non-invasive, longitudinal monitoring of HCC progression with high sensitivity and reliability. With easy setup and no contrast agent or respiratory triggering required, MDEFT/RARE provides a powerful alternative for accurate quantification of HCC, with relatively high throughput, improved study power, and reduction in animal use.

References:

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