MR Vessel imaging for Tumor Angiogenesis Quantification in Two Rodent Models of Hepatocellular Carcinoma

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Introduction
Hepatocellular carcinoma (HCC) are typically hypervascular tumors. Angiogenesis, the process by which new vessels develop from pre-existing microvessels, plays an important role in HCC progression. Experimental and clinical studies indicate that patients with higher microvesSEL density in tumors have a poor prognosis, hence angiogenesis is regarded as an important bio-marker (1). MR vessel imaging methods, using intravascular ultrasmall superparamagnetic iron oxide (USPIO) contrast agents, has been developed to monitor tumor angiogenesis (2). Vessel size index (VSI) was calculated based on the changes of magnetic relaxation parameters induced with USPIO agent, is independent of imaging resolution. The aim of our study was to evaluate MR vessel imaging techniques for tumor angiogenesis quantification in rat models of HCC. Two different rodent HCC models were chosen: McA-RH7777 Morris hepatoma model and N1-S1 hepatoma model.

Materials and Methods
Animal Models
All studies were approved by our institutional animal care and use committee and were performed in accordance with institutional guidelines. 16 adult male Sprague Dawley rats (Charles River Laboratories, MA, USA) were used for these experiments. After anesthesia, a mini-laporatomy was performed and the left hepatic lobe exposed on a sterile transport. 5 × 10⁷ McA-RH7777 rat hepatoma cells were visually injected into the left medial hepatic lobe in 7 rats and 5 × 10⁷ N1-S1 rat hepatoma cells were injected using the same procedure in remaining 9 rats. 7 days were allowed for tumor growth and angiogenesis after initial implantation.

MRI
All MRI studies were performed using a 3.0T Magnetom Trio Clinical scanner (Siemens Medical Solutions, Germany) with custom-built rodent receiver coil (Chengdu Medical Technologies, China). The rats were anesthetized with a high-limb injection of ketamine and xylazine. Three to five contiguous 3-mm-thick axial slices were imaged to ensure the complete coverage of the entire tumor. Five datasets were acquired for each animal. First, an ADC map was calculated using the single-shot DW-SE-EPI sequence with the following 6 b-values: 0, 50, 100, 200, 300, and 500 sec/mm². Other imaging parameters were: TR = 3 s, TE = 78 ms, FOV = 120 mm, 128 × 64 matrix, BW = 1500 Hz/pixel, 6 averages. DW images were acquired during free-breathing using respiratory triggering. Next, single-echo SE sequences were used to calculate T2 maps with 6 TEs (TR = 2 sec, TE = 10, 20, 30, 40, 50 and 60 ms, 4 averages). Then a MGRE sequence was used to calculate T2* map (TR = 150 ms, ETL = 12, ES = 3.06 ms). Following pre-contrast T2* mapping, 1.1 ml/kg of USPIO contrast agent (200 µmol of iron/kg, Molday ION; BioPal, Worcester, MA) was injected via a femoral vein catheter, followed 1 ml saline. After 2 min to allow the contrast agent to circulate, the SE and MGRE scans were repeated to acquired post-contrast T2 and T2* maps.

Data Analysis
Voxel-wised ADC, T2 and T2* maps were calculated by employing the non-linear Levenberg-Marquardt algorithm to fit the mono-exponential function. Blood volume fraction (BVF) and VSI were calculated according to the following equations:

\[ \text{BVF}_{\text{USPIO}} = (3/4\pi)(\Delta R/\Delta R_0) \text{, } \text{VSI}_{\text{USPIO}} = 0.425(\text{ADC}/\gamma_2 B_0)^{1/2}(\Delta R/\Delta R_0)^{1/2} \]

where \( R_2 = 1/T2, R_2^* = 1/T2^* \), \( \Delta R_2 = R_2^* - R_2^* \text{pre-contrast}, \Delta R_2^* = R_2^* - R_2^* \text{post-contrast}, \) and \( \Delta \chi \) is the susceptibility difference between blood and tumor tissue in the presence of the intravascular USPIO contrast agent.

Histology
CD34 staining was used as a reference standard to quantify tumor angiogenesis. Histology slices were digitized. A binary mask was created by thresholding the gray level of the blue channel. Within the whole tumor, vessel objects were detected and an area was measured (number of enclosed pixels). A mean BVFUSPIO was calculated as the total vessel area divided by tumor area. A mean BVFUSPIO was calculated as the total vessel area divided by tumor area. A mean vessel radius was calculated as described in prior study (3). The relationship between tumor VSI and USPIO measured BVF with MRI USPIO imaging techniques and histology was assessed by calculating Spearman correlation coefficients. Mean VSIUSPIO and BVFUSPIO from the two tumor models were also compared using independent sample t-test.

Results
The mean ADC of McA-RH7777 and N1-S1 hepatoma models was 0.84 ± 0.11 × 10⁻³ mm²/s and 0.57 ± 0.02 × 10⁻³ mm²/s. The ADC values within the McA-RH7777 tumors were significantly higher than the ADC values within the N1-S1 tumors (p < 0.01). The representative BVFUSPIO and VSIUSPIO maps for the McA-RH7777 and N1-S1 tumors are shown in Fig 1. Fig 2. shows the representative CD34 staining for McA-RH7777 and N1-S1 tumors. Blood vessels were stained brown in the tumor region (blue background). McA-RH7777 tumors were more vascular than N1-S1 tumors. The mean BVF measured by MRI within the McA-RH7777 tumors was significantly larger compared to the value within the N1-S1 tumors (p < 0.01); while the VSI within the McA-RH7777 tumors was significantly lower than the values within N1-S1 tumors (p < 0.05). MRI measured tumor angiogenesis parameters (VSI and BVF) were compared to histology angiogenesis measurements, shown in Fig 3. Statistical correlation was observed between BVFUSPIO and BVFUSPIO measurements (r = 0.758, p < 0.01).

Conclusion
We observed a significant correlation between the MRI BVF and VSI measurements and the results from CD34 histology staining in two rodent HCC models. MR vessel imaging using USPIO contrast agents may potentially serve as a non-invasive method to evaluate tumor angiogenesis during tumor progression in HCC.

Fig 1. Strong correlations were observed VSIUSPIO and VSIUSPIO (a) and between BVFUSPIO and BVFUSPIO (b)

Fig 2. Representative CD34 staining for McA-RH7777 (a) and N1-S1 (b) tumors. McA-RH7777 tumors were more vascular than N1-S1 tumors.

Fig 2. Representative BVF (a) and VSI (b) maps measured with USPIO MRI for McA-RH7777 (Group 1) and N1-S1 tumors (Group 2).

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