Gas challenge-blood oxygen level dependent (BOLD) MRI in monitoring tumor angiogenesis of a rodent Novikoff hepatoma model

Y. Guo[1], N. Jin[1,2], R. Klein[1], G-Y. Yang[3], R. Omary[1,2], and A. Larson[1,2]
[1]Department of Radiology, Northwestern University, Chicago, IL, United States, [2]Department of Biomedical Engineering, Northwestern University, Chicago, IL, United States, [3]Department of Pathology, Northwestern University, Chicago, IL, United States

INTRODUCTION

Angiogenesis is an important factor affecting the rate of tumor metastasis and growth; the potential efficacy of anti-angiogenic oncologic therapies has been demonstrated in both pre-clinical and clinical trials (1). Non-invasive methods to monitor tumor neo-vascular changes during tumor progression and/or in response to anti-angiogenic therapy may be critical. Blood oxygenation level dependent (BOLD) MRI uses deoxyhemoglobin (deoxyHb) levels in tissue as a biomarker of oxygenation, blood volume, and perfusion. Prior studies have demonstrated the feasibility to use BOLD-MRI to monitor tumor growth rate and tumor vasculature in a number of different animal models (2-4). The purpose of our study was to investigate the relationship between gas-challenge (GC)-BOLD response and degree of tumor angiogenesis during tumor size progression in the rodent N1-S1 hepatoma model.

METHOD

Animal Model 11 adult male Sprague Dawley rats (weighting 301-325g) were used for our ACUC-approved experiments. $1x10^6$ N1-S1 rat hepatoma cells (ATCC, Manassas, VA) were implanted in the left medial hepatic lobe; 8 rats developed hepatoma sizes 0.72cm to 2.81cm. *MRI* Rats were anaesthetized with ahigh limb injection of ketamine (75-100mg/kg) and xylazine (2-6mg/kg). All experiments were performed using a 3.0T clinical MR scanner (Magnetom Trio, Siemens) with custom rodent receiver coil (Chenguang Med. Tech. Co., Shanghai, China). Coronal and transverse T2-weighted TSE images of the entire liver were acquired for localization. 3-5 axial slices passing through the N1-S1 hepatoma were selected for our BOLD studies. For R2* measurements we used a multiple gradient-echo (MGRE) sequence with parameters: TR=150ms, ETL=12 (4ms spacing), FA=30º, 3mm slices, 150mm FOV, 192 matrix, averages = 25. Room air (78% N2/20% O2) or carbogen (95%O2/5%CO2) was administered via a rat nose-cone. MGRE images were first acquired during air breathing; then the animal was given carbogen for ten minutes for transition, and a second set of MGRE images were acquired while the animal continued to breathe carbogen. After image acquisition, rats were euthanized and the tumors were harvested for histological evaluation.

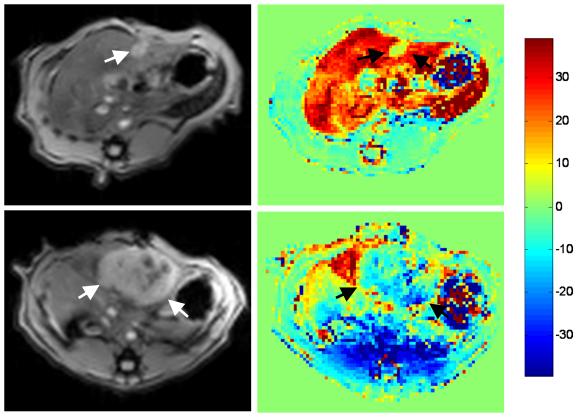

Fig. 1 MGRE images (**left,** TE=12ms) and corresponding ΔR2* maps (**right,** scale 1/s) for 2 N1-S1 hepatoma (arrow)

Images Analysis R2* maps were calculated by employing the nonlinear Levenberg-Marquardt algorithm to fit the mono-exponential function $S(TE_i) = S(0)\cdot\exp(-R2^*\cdot TE_i)$ using Matlab software (The Math Works Inc., Natick, MA). R2* change maps were calculated as R2* air – R2* carbogen. For each animal a region of interest (ROI) was drawn in the hepatoma to measure mean R2* change values. Tumor size measurements were performed with the maximum lesion diameter measured within T2W-TSE images (5).

Histology Hepatoma specimens were fixed in formalin and paraffin embedded. CD34 staining was used to identify angiogenesis within the tumors (6). Histological slides were digitized with x100 optical magnification using a multi-channel automated imaging system. A quantitative assessment of tumor microvessel density was performed to quantify the total CD34 stained vessel areas per one thousand tumor pixel area.

Statistical Analysis All statistics were performed using SPSS (SPSS, Chicago, IL, USA). The Spearman's correlation coefficient was calculated to assess the correlation between the tumor R2* change and tumor microvessel density and tumor size. Test was considered statistically significant with a p-value < 0.05.

RESULTS

R2* change between air and carbogen breathing for small hepatoma (0.8cm diameter, top row) were positive and progressed to negative for larger hepatoma (2.8cm diameter, bottom row) (**Fig. 1**). Pathology specimens demonstrated diffuse CD34 expression in the tumor region highlighting sinusoidal capillarization (**Fig. 2**). During tumor progression (increasing angiogenesis levels and size) we found a significant positive correlation between tumor R2* change and tumor microvessel density (r = 0.902, p = 0.003) and a significant inverse correlation between tumor R2* change and tumor size measurement (r = -0.802, p = 0.017) (**Fig. 3**).

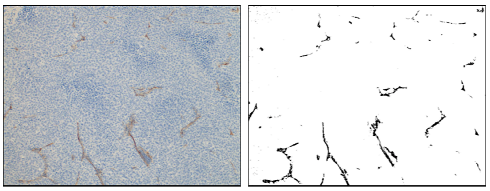

Fig. 2 CD34 staining showing diffuse positive CD34 expression (stained brown, **left**) and color differentiation of CD34 (black, **right**) for quantification of tumor microvessel density.

CONCLUSION

Angiogenesis is fundamental for tumor growth, invasion and metastasis. In this study, a positive correlation was found between GC-BOLD response and tumor microvessel density and a negative correlation was between GC-BOLD response and tumor size. GC-BOLD MRI may offer the potential to serve as a non-invasive method for evaluating angiogenesis and monitoring anti-angiogenic therapy response in hepatic tumors.

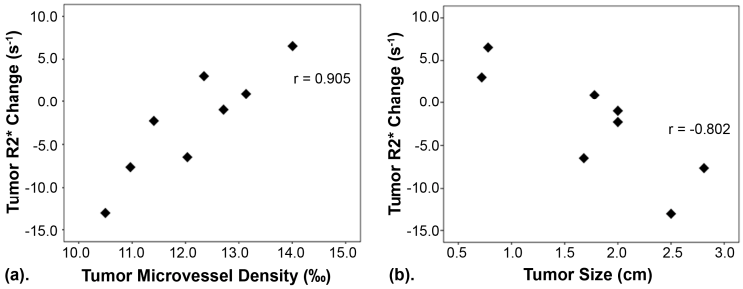

Fig. 3 Scatter-plots comparing (a) tumor microvessel density and (b) tumor size to tumor R2* change. A significant positive correlation observed for tumor microvessel density (r = 0.902, p = 0.003) and inverse correlation observed for tumor size (r = -0.802, p = 0.017).

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