Steady-state susceptibility contrast MRI detects early anti-angiogenic effects of a novel biomimetic peptide in a human breast cancer model

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Target audience: This work is targeted toward researchers and clinicians interested in noninvasive biomarkers of anti-angiogenic treatment response in cancer.

Purpose: There is a critical need for both improved anti-angiogenic therapies and noninvasive biomarkers of early anti-angiogenic treatment response. Steady-state susceptibility contrast (SSC)-MRI is a clinically translatable technique that is sensitive to changes in blood vessel morphology[1,2]. The aim of this study was to use SSC-MRI to evaluate the efficacy of a novel biomimetic anti-angiogenic peptide that we developed[3] in an orthotopic human breast cancer model. Our hypothesis was that SSC-MRI provides biomarkers of early anti-angiogenic treatment response that are more sensitive than conventional markers of therapeutic efficacy like tumor volume and cellularity.

Methods: MDA-MB-231 human breast cancer cells were orthotopically inoculated into the mammary fat pad of 10 female athymic nude mice. After the tumors reached ~70 mm^3, they were imaged in vivo on a Bruker Biospin 9.4T small animal MRI system using the following sequences: (i) 2D diffusion-weighted spin echo, echo time (TE) = 26.6 ms, repetition time (TR) = 1000 ms, b-value = 0 and 327 s/mm^2, and three orthogonal diffusion-sensitizing gradient orientations. (ii) 2D multiple gradient echo (MGE), TE = 5 ms, six echoes with 5 ms echo spacing, TR = 800 ms. (iii) 2D multiple spin echo (MSE), TE = 10 ms, eight echoes with 10 ms echo spacing, TR = 1500ms. MGE and MSE images were acquired before and five minutes after i.v. injection of ferumoxytol (5 mg Fe/kg). For all sequences, in-plane resolution = 125 μm, slice thickness = 1 mm, and the number of slices varied to cover the entire extent of each tumor. The scan time for each mouse was ~1 h. Starting on the day of imaging (day 0) after imaging was completed, five mice were treated daily for 14 days with the anti-angiogenic peptide SP2024 (10 mg/kg), while the other five served as vehicle-treated controls. The tumors were imaged again with the same protocol on day 14. Pre- and post-treatment apparent diffusion coefficient (ADC) and SSC-MRI vascular biomarker maps were computed as described in [4]. SSC-MRI biomarkers included FBV (fractional blood volume), VSI (vessel size index), and Q (vessel density). The changes in tumor volume and median MRI parameter values from day 0 to day 14 were computed. After the post-treatment scans, the tumors were excised, fixed, frozen and cut for H&E and immunofluorescent staining of laminin (an endothelial basement membrane protein). H&E and laminin images were binarized to quantify viable tumor fraction and vascular area fraction, respectively. Two-tailed Mann Whitney-U tests (α=0.05) were used to test for differences between control and treated tumors.

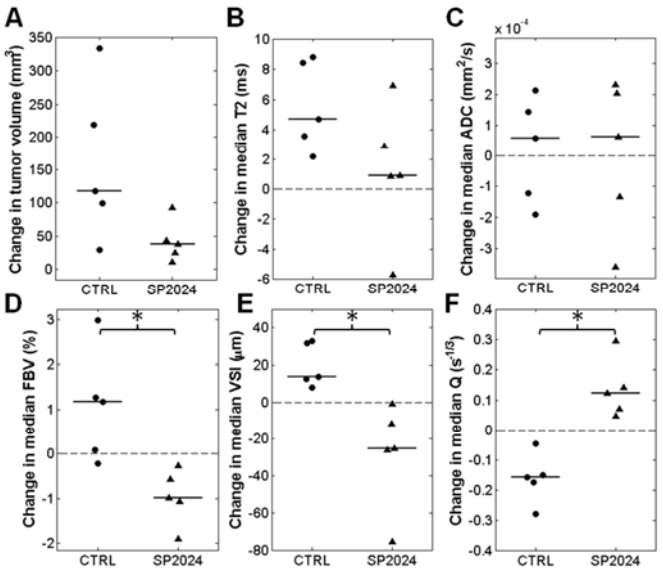

Fig 1 Plots of changes in tumor volume (A) and median MRI parameter values (B-F) from day 0 to 14 for individual control (CTRL) and treated (SP2024) tumors. Solid horizontal lines indicate group medians. *p<0.05

Results: SP2024 treatment resulted in reduced tumor growth, but the changes in tumor volume were not yet significantly different between treated and control tumors by day 14 (p=0.06, **Fig 1A**). There were also no significant differences in the changes in pre-contrast T2 (p=0.10) or ADC (p=1) (indicators of cellularity) between the two groups (**Fig 1B,C**), which was validated by demonstrating that there was no difference in viable tumor fraction calculated from H&E images (**Fig 2A-C**). In contrast, the changes in SSC-MRI vascular parameters were significantly different between treated and control tumors (**Fig 1D-F**) at this early stage. Median FBV and VSI decreased while Q increased in all treated tumors, whereas the opposite trends were observed for the control tumors. This SSC-MRI-measured anti-angiogenic treatment effect was confirmed by the reduced vessel calibers and vascular area fraction determined from images of laminin-stained tumor sections (**Fig 2D-F**).

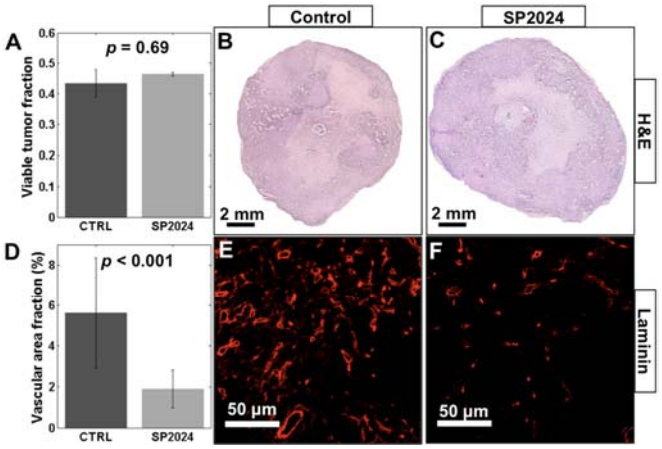

Fig 2 A) Viable tumor fraction computed from H&E images (B,C). D) Vascular area fraction computed from fluorescently labeled laminin images (E-F).

Discussion: Response Evaluation Criteria in Solid Tumors (RECIST) is commonly used to evaluate the efficacy of anti-angiogenic drugs, but there may be a delay between an anti-angiogenic response and a detectable change in tumor size[5]. Such was the case in this study – SP2024-treated tumors saw marked decreases in vascularity but did not differ in size from control tumors. Although SP2024 has been shown to trigger apoptosis in this tumor model, there was no difference in cellularity between treated and control tumors at this early stage (i.e. 2 weeks). However, the vasculature-specific SSC-MRI parameters were sensitive to treatment-induced inhibition of angiogenesis before the manifestation of other measureable therapeutic effects. This suggests that SSC-MRI is able to provide biomarkers of early response to anti-angiogenic therapy and thus may aid in evaluating the efficacy of new drugs and optimizing treatment strategies.

References: 1. Jensen and Chandra, *Magn Reson Med* 2000;44(2):224-30. 2. Troprès et al., *Magn Reson Med* 2001;45(3):397-408. 3. Rosca et al., *Chem Biol Drug Des* 2012;80:27-37. 4. Kim et al., *Magn Reson Med* 2013;70(4):1106-16. 5. Lindner and Borden, *Int J Cancer* 1997;71:456-461.