

Intratumor and Intertumor Heterogeneity in Contrast Agent Kinetics as Assessed by Functional MRI - Initial Results with Implications for Metastasis

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SYNOPSIS: While MRI has been applied to study tumor angiogenesis, its application to studying lymphatic dissemination and lymphangiogenesis has been limited at best. In this study, we characterized early and late changes in the distribution kinetics of albumin Gd-DTPA in a solid tumor mouse model, with and without an implanted vascular endothelial growth factor (VEGF) pellet. We demonstrated that lymphatic and convective conduits for tumor cell propagation could be detected with MRI, and histologically confirmed the same using a novel lymphatic endothelium marker (LYVE-1). Our initial results suggest a role for VEGF in lymphatic metastasis.

INTRODUCTION: Metastases can occur via vascular as well as lymphatic dissemination. Although lymph node metastasis is one of the most powerful indices for prognosis, particularly in breast cancer, mechanisms underlying this phenomenon have largely been eclipsed by the focus on angiogenesis and vascular dissemination. While MR methods have been applied to study the tumor vasculature, their application to studying lymphatic drainage and interstitial clearance has been limited at best. In this study we have characterized the early and late changes in the distribution kinetics of a macromolecular MR contrast agent (albumin Gd-DTPA) in a solid tumor model both, with and without an implanted VEGF pellet. Our goal was twofold: (i) to determine if potential lymphatic or convective conduits could be detected with MRI, and (ii) to assess the role of VEGF in (lymphatic) metastasis. In addition, lymphatic endothelium in tumor sections was identified using LYVE-1 staining [1].

METHODS: We conducted MR measurements on MDA-MBA-231 tumors inoculated in the mammary fat pad of nine mice. A slow release (1.6 μ g over 21 days) VEGF pellet (Innovative Research, Sarasota, FL) was transplanted adjacent to the tumor in five of the mice once the tumor reached 200mm³ in volume. The four remaining mice received a placebo pellet. Multi-slice MR T1 relaxation rates of the tumor were obtained by a saturation recovery method combined with fast-T1 SNAPSHOT FLASH, which provided images of the entire mouse cross-section including the tumor. Four, 1mm slices were acquired with a 256 $\times$ 256 μ m² resolution for three relaxation delays (100, 500 and 1000ms). Images were obtained before i.v. administration of 0.2ml of albumin Gd-DTPA and repeated every 5 min, starting at 3 min post-injection, up to 30 min, followed by an interval of one hour, after which a second block of data was acquired up to 180 min. Image time courses corresponding to the 1s relaxation delay were analyzed using cross-correlation (cc) analyses to identify voxels that had a shape similar to that of a pre-selected reference waveform that would best represent convective or lymphatic contrast agent kinetics [2].

RESULTS: Fig. 1a (152 min post-contrast) shows enhancing channels and pellet site. MRI voxels with at least three distinct contrast signatures were detected with high confidence (cc>0.8): group (i) potential convective/lymphatic channels (Fig. 1b'), (ii) normal tissue outside tumor (Fig. 1c'), and (iii) potential necrotic areas (Fig. 1d'). Overall there seemed to be more group (i) voxels in the VEGF animals compared to the placebo animals. These data suggest that VEGF increased the number of conduits as detected by MRI. In a separate study [3], we measured the kinetics of Albumin-Gd and observed a small decrease in R1 of blood over the duration of a typical MRI protocol (Fig. 2). The increases in MR signal identified in "late" signal enhancement voxels (Fig. 1b') was therefore not related to blood R1 changes. Finally, our results from immunostaining of tumor sections with the lymphatic endothelium-specific receptor LYVE-1 [1], indicated the presence of lymphatics (Fig. 3) in the tumor periphery, consistent with the appearance of several, but not all MRI detected convective/lymphatic voxels located in the tumor periphery.

DISCUSSION/CONCLUSIONS: These data suggest that in keeping with its known role as a permeability enhancer, VEGF increases vessel permeability in the tumor. The resulting extravasation of contrast agent and macromolecules into the tumor interstitium will lead to increased convection and potential drainage via lymphatic channels, which would appear as a late event in the MRI data. The identification of channels both via MRI and histology has the potential to increase our understanding of the mechanistic aspects of tumor metastasis. Currently, we are in the process of improving our contrast kinetics detection algorithm to quantify differences between the two groups, and identify vascular and lymphatic pathways histologically. Finally, we are in the process of determining trends in the vascular volume and permeability surface area measured with MRI [4], to better understand the role of VEGF in lymphangiogenesis.

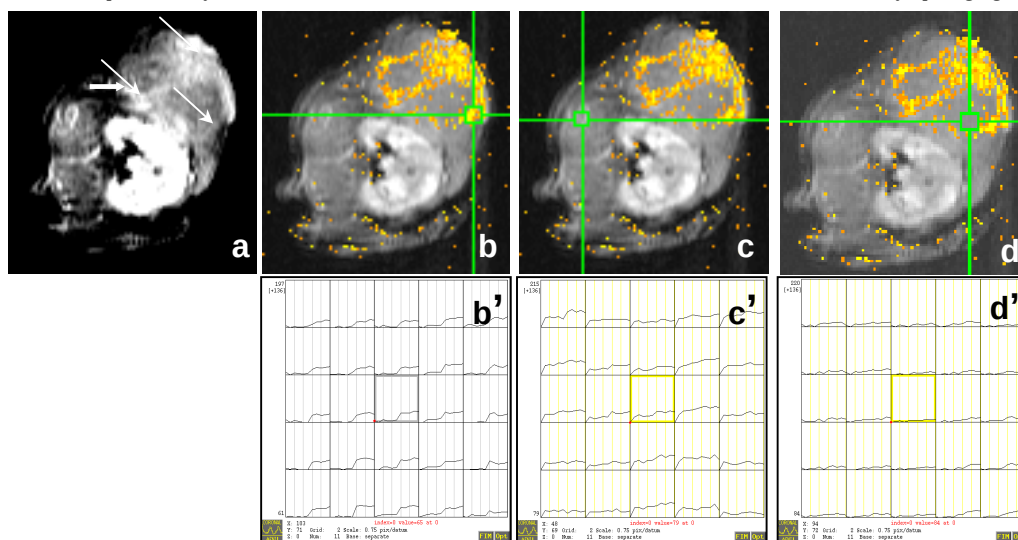


Fig. 1 MR images: (a) 152 min post-contrast showing enhancing channels ($\rightarrow$) and location of VEGF pellet ($\rightarrow$); (b) correlation coefficient map (cc) overlay (>0.8) and (b') signal time courses for a conduit ROI (in cross-hairs); (c) the same cc overlay (>0.8) and (c') signal time courses for a normal tissue ROI (in cross-hairs); (d) the same cc overlay (>0.8) and (d') signal time courses for a necrotic ROI (in cross-hairs).

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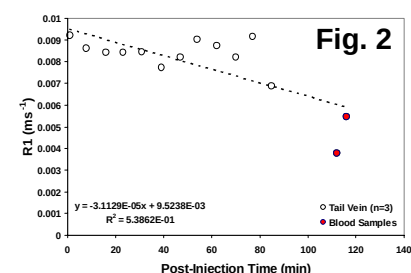


Fig. 2 Kinetics of Albumin-Gd in the blood of mice bearing MDA-MB-231 tumors.

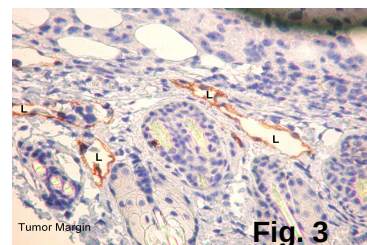


Fig. 3 Magnified (40 $\times$) 5 μ m tumor section stained with LYVE-1 and counterstained with hematoxylin. Lymphatics (L) are easily identifiable in the tumor margin.