Characterizing Extravascular Fluid Transport of Macromolecules in the Tumor Interstitium by MRI

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INTRODUCTION: The ability to image and characterize the transport of macromolecular agents through the ECM can be employed to understand factors affecting macromolecular transport, and cancer cell dissemination. In normal tissue, macromolecules and cell debris are cleared from the interstitium via lymphatic drainage. In tumors, lymphatics have either been shown to be poorly developed or non-functional due to the existing mechanical stresses, with the result that the transport of macromolecules through the ECM is thought to occur primarily via convection [1]. Here, we demonstrate for the first time, the ability of MRI of the macromolecular contrast agent (MMCA) albumin-GdDTPA to quantify delivery, transport rates and volumes of macromolecular fluid flow through the interstitial-lymphatic continuum in tumors. Fluorescent microscopy of LYVE-1 (lymphatic vessel endothelial hyaluronan receptor), isolectin BS-I-B4 (a vascular endothelial cell specific marker), and biotinylated-albumin-GdDTPA was performed to determine the presence and contribution of lymphatic vessels to macromolecular drainage within the tumor.

METHODS: MRI of five anesthetized MCF-7 tumor-bearing (>200mm3) mice was conducted. Multi-slice T1 relaxation rates of the tumor were obtained by saturation recovery combined with SNAPSHOT FLASH. Eight (1mm) slices were acquired with a 256×256μm^2 resolution for three relaxation delays (100, 500 and 1000ms). Images were acquired in two "phases" corresponding to the biphasic kinetics of the MMCA (**Fig. 1**). The "early phase" comprised of images obtained before i.v. administration of 0.2ml of 60mg/ml albumin Gd-DTPA (or biotinylated-albumin-GdDTPA) saline and repeated every 7 min, starting at 3 min post-injection, up to 31 min (**Fig. 1c**). Since drainage of macromolecules in and around tumors either by convection or by lymphatics, is a slow event [2], a second block of MR data was acquired up to 140 min post contrast. This acquisition was classified as the "late-phase" of the MMCA, as it consists of late drainage events within the ECM of the tumor (**Fig. 1d**). After each study, mice were sacrificed and blood T1's determined from tail vein samples. Parameters describing vascular and extravascular transport of MMCA in MCF-7 tumors were calculated from the MMCA tissue concentration-time curves ($\Delta R1_{Tissue}(t)/\Delta R1_{Blood}(t)$), by assuming three compartments within the tumor:(i) intravascular space, (ii) perivascular region of the tumor interstitium that the MMCA initially extravasates into, and (iii) a more distant compartment consisting of regions of ECM within which slow macromolecular transport events such as convective and lymphatic drain occur (**Figs. 1a, b**). The two phases of the MR concentration-time data were analyzed using a novel multiple regression approach [3] that allowed us to compute: (i) vascular volume and permeability-surface area product (**Fig. 1 c**) from the *early phase*, (ii) the macromolecular fluid exudate volume and influx/efflux rates from the *late phase*, and (iii) it enabled us to identify voxels exhibiting either contrast pooling or voxels from which contrast was being drained from comparison of the regression parameters between the *late*- and *early-phases* (**Fig. 1d**). Tumor sections were stained for lymphatic vessels (**LYVE-1**), blood vessels (**isolectin BS-I-B4**) and the MMCA (**biotinylated-albumin-GdDTPA**).

RESULTS: Average VV and PS of MCF-7 tumors was 4.60±2.1μl/g and 0.43±0.19μl/g.min, respectively. Overall, there was a greater percentage of pooling voxels (19.9±16.8) identified compared to draining voxels (2.9±1.5) (**Fig. 2a**), with pooling voxels often found in proximity to draining voxels (**Fig. 2b**). The influx rate (1.31±0.28μl/g.min) was significantly (p=0.001) greater than the efflux rate (-0.28±0.17μl/g.min) for all imaged tumors. Although PS of the draining regions (0.87±0.54μl/g.min) tended to be higher than that of the pooling regions (0.25±0.16μl/g.min), this difference was not significant. In contrast, VV of the pooling regions (7.68±1.55μl/g) was significantly (p=0.001) higher than that of the draining regions (3.68±1.88μl/g). Finally, there was a significant difference (p=0.001) in the apparent exudate volume of the pooling voxels (36.63±7.70μl/g) compared to the draining voxels (-7.90±4.72μl/g). **Fig.2b** illustrates MMCA drain occurring at the tumor-host tissue interface (arrows). Lymphatic vessels (green) were observed in the tumor margins with virtually no overlap with blood vessels (red). Almost no intratumoral lymphatics were detected in the central regions of tumors. At 100×, sparsely distributed lymphatics in the tumor periphery were often seen to be intussuscepted by tumor cells (**Figs. 3a, 3b**). **Fig. 3c** illustrates the intratumoral distribution of biotinylated albumin-GdDTPA (blue) in which several MMCA-bearing blood vessels are apparent (arrows), including areas with extravasated MMCA. In contrast, no MMCA-bearing lymphatic vessels were detected in any tumor section.

DISCUSSION/CONCLUSIONS: Pooling regions exhibited higher exudates volume than draining regions, as well as a higher (in)flux rates compared to the draining voxels, consistent with the presence of a significant fraction of hyperpermeable vessels. Total MR detectable interstitial fluid volume an hour after onset of the late phase was comparable to the elimination rate of ~0.2ml/hr reported by others [4] for tumor interstitial fluid. The smaller exudate volume together with a lower (ef)flux rate of MMCA from draining voxels demonstrates that the rate and amount of macromolecular clearance from the tumor interstitium is low, suggesting that clearance of macromolecules from the ECM was not efficient, consistent with the few lymphatic vessels detected in these tumors. Although, immunofluorescence revealed the presence of a small number of lymphatic vessels mostly localized to the tumor periphery, consistent with the drainage ROIs identified by the MRI analyses, there was no co-localization of biotin-labeled MMCA with the LYVE-1 stain. Thus, involvement of peripheral lymphatics in MMCA clearance for this tumor type was most likely minimal. The low fractional area of LYVE-1 stained lymphatics in comparison to that of the draining ROIs identified using MRI, implies that most of the interstitial MMCA drainage for MCF-7 tumors was by convection and not via lymphatics. The dynamic, noninvasive, *in vivo* functional assay described here, provides a powerful tool for probing the interstitial-lymphatic continuum with the potential to increase our understanding of the mechanistic aspects of tumor metastasis.

REFERERENCES: **1.** Netti PA et al. *Cancer Res* 2000;60:2497-503. **2.** Dafni H et al. *MRM*(50): 904-914,2003. **3.** Pathak AP et al. *Cancer Res* 2004 (submitted). **4.** Butler TP et al. *Cancer Res* 1975;35:3084-8. **ACKNOWLEDGEMENTS**: Supported by NIH RO1 CA90471. We thank Mr. G. Cromwell for transplanting tumors and cell work.

Fig. 1

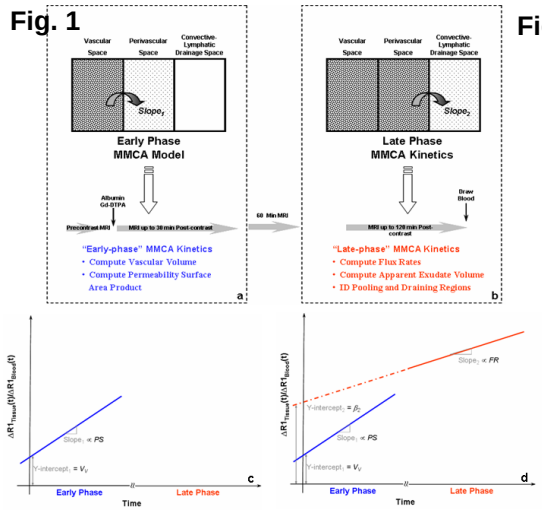

Fig. 2

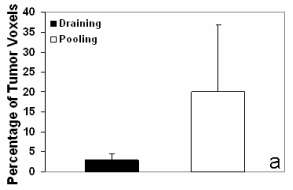

Fig. 3