High-Resolution 3D MRI Mapping of Tumor Angiogenesis using $\alpha_5\beta_1$-Targeted Perfluorocarbon Nanoparticles

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Introduction: One important receptor in developmental angiogenesis and pathological tumor neovasculature is $\alpha_5\beta_1$–integrin. $\alpha_5\beta_1$–integrin and its ligand fibronectin are coordinately up-regulated on neovessels in tumor biopsies; blockade of this receptor by antibody or small molecule inhibits angiogenesis (1). While other molecular imaging modalities detect the presence biochemical epitopes, MRI can provide unique, visualization and quantification of the 3D distribution of these biosignatures. The objectives of this study were to develop an $\alpha_5\beta_1$–targeted paramagnetic nanoparticle; to image the up-regulation of $\alpha_5\beta_1$–integrin expression in xenograft MDA 435 tumors in mice at 1.5T; and to characterize the 3D distribution of $\alpha_5\beta_1$–integrin positive vasculature around the implant.

Materials and Methods: Paramagnetic perfluorooctylbromide (PFOB) nanoparticles were made as previously published (2) using 30% (w/v) Gd-DOTA-NH2-PE attached to the outer surface membrane and targeted to the $\alpha_5\beta_1$-integrin using a cyclic peptide ligand (ACRGDGWCG, IC_{50} ~6μM).(3) The bioactivity of $\alpha_5\beta_1$–targeted nanoparticles was confirmed using an HT1080 cell adhesion bioassay immobilized to fibronectin.

Nude mice implanted with human MDA 435 cells into the caudal mammary fat-pad 14 days previously were administered paramagnetic control (n=6) or $\alpha_5\beta_1$-targeted nanoparticle (n= 7) (IV) and dynamically imaged by MRI (Philips Gyroscan NT Intera, 1.5T) at baseline, 30, 60, 90, and 120 min. post-injection. High-resolution, 3D, T1-weighted, fat suppressed gradient echo images (FOV 55mm, 0.5 mm slices, 256x256 matrix, TR/TE = 46/5.8 ms, 65° flip angle) were collected using a commercially-available 47mm circular surface coil. Contrast-enhanced voxels at 120 minutes were selected based on a threshold equal to three times the standard deviation of the tumor signal at baseline. The change in signal of these voxels was then calculated for each previous time point. The area of enhancement, the average signal increase, and the contiguity of enhancing voxels in 3D space were determined. The relative distribution of enhancing voxels in the periphery versus the core of the tumor was estimated by 3D MRI and supported by imaging and co-registration of fluorescent nanoparticles with lectin stained vasculature.

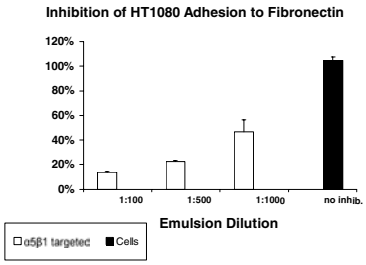

Fig 1. white bars -'$\alpha_5\beta_1$-targeted emulsion + cells'; black bars-'cells alone'

Results $\alpha_5\beta_1$–targeted paramagnetic nanoparticles (308nm, -56.9mV at pH 7) were developed and found to inhibit HT1080 cell adhesion to fibronectin as a monotonic function of dilution (Fig.1). Intravenous administration of these nanoparticles to nude mice bearing MDA 435 tumors resulted in increased MR T1w signal enhancement of the targeted tumors versus control ($p<0.05$), which was best reflected as the product of the voxel number multiplied by signal enhancement, (i.e. the contrast index, Fig 2.) Approximately 90% of the enhanced targeted voxels were located in the periphery of the tumor and comprised 2.0% ± 0.5% of the rim area; the remainder was scattered within the core. In the $\alpha_5\beta_1$–targeted tumors, clusters with up to 100 adjacent voxels were contrast-enhanced; however, in the control animal tumors, signal enhanced voxels were generally noncontiguous, with the largest grouping comprised of 5 voxels in one nontargeted tumor. $\alpha_5\beta_1$–immunohistochemistry demonstrated the presence of $\alpha_5\beta_1$–integrin in extravascular tissues; whereas, $\alpha_5\beta_1$–fluorescent nanoparticles were associated with vasculature co-stained by intravenous injection of FITC-lectin. Although the variability of angiogenesis micromorphology was suggested by histology, MR 3D mapping of the $\alpha_5\beta_1$–positive neovasculature better revealed and quantified the heterogeneous distribution of neovessels around the tumors.

Discussion In this study, we have demonstrated a new paramagnetic nanoparticle formulation targeted to $\alpha_5\beta_1$–integrin positive neovasculature. Histology studies confirmed that the $\alpha_5\beta_1$–nanoparticles were associated with the vasculature and did not reach $\alpha_5\beta_1$–integrin expressed by cells in the extravascular matrix. 3D mapping of $\alpha_5\beta_1$–integrin positive neovasculature characterized the heterogeneous distribution of angiogenesis, which was found predominantly around the periphery of the tumor . These results illustrate the unique opportunities of MRI molecular imaging to spatially delineate, characterize and quantify the expression of neovascular biomarkers associated with cancer.

References 1. Kim S et al. J. Am J Pathol 2000;156(4):1345-1362. 2. Schmieder A et al. Magn Reson Med 2005;53(3):621-627. 3. Koivunen E Biotechnology 1995;**13**(3): 265-270.

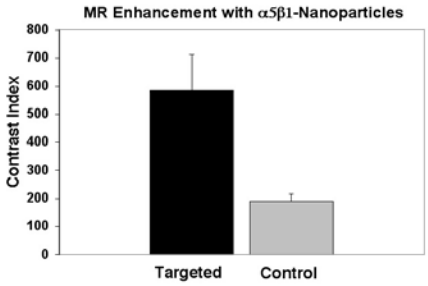

Fig 2. Contrast Index at 120 min.

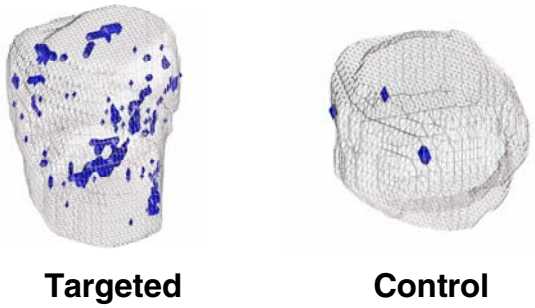

Fig 3. 3D reconstruction of MR contrast enhancement