Dual PI3K/mTOR Inhibition Induces Structural Changes in Tumor Vasculature Assessed by Vessel Size Imaging

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Objectives. Previous studies have reported a reduction in the dynamic contrast enhanced magnetic resonance imaging (MRI) parameter K^{trans} following treatment with a dual PI3K/mTOR inhibitor (PI3K/mTORi) [1]. These K^{trans} changes were attributed to an inhibition of vascular endothelial growth factor signaling through PI3K, leading to a suppression of eNOS-induced vascular permeability and vasodilation [1]. However, the effects of PI3K/mTORi on vascular structure remain unknown. This study aims to elucidate the role of PI3K and mTOR inhibition on vascular structure using an *in-vivo* multispectral vessel size index (VSI) MRI approach and *ex-vivo* micro-computed tomography (μCT) angiography.

Methods. *Animal Model.* Animal procedures were approved by the institutional AAALAC-accredited review board. Two cohorts of athymic nude mice were inoculated subcutaneously on the hind limb with $3.5x10^6$ HM7 (human colorectal cancer) cells for the independent VSI and μCT studies. *Multispectral VSI MRI*: MRI was performed on a 4.7T Varian Unity Inova MRI system with a Varian 20mm two-loop surface coil. Eight coronal, 1-mm-thick slices were acquired with a 25.6x25.6mm FOV and 64x64 (ADC, T_2) or 128x128 (T_2*) matrix. A multi-slice, diffusion-weighted fast spin-echo imaging sequence was used to obtain ADC measurements (6 b-values from 82-1129 s/mm^2, TR=3s, ETL=4, NEX=2, δ=3.3ms, Δ=30ms). T_2 and M_0 maps were acquired using a multi-slice, spin-echo imaging sequence (sems, TE=5,26,47,68 ms, TR=3s and NEX=1) and T_2* maps were acquired using a multi-echo multi-slice gradient echo sequence (mgems, TE=5,10,15, 20,25,30,35,40ms, TR=345ms and NEX=4). Subsequently, a USPIO contrast agent (200μmol/kg, Molday ION, BioPAL) was delivered via tail-vein catheter and post-contrast sems and mgems sequences were repeated to calculate T_2 and T_2* maps, respectively. Multispectral VSI MRI parameters including vessel density (Q), VSI, and fractional blood volume were calculated voxel-by-voxel in the viable tumor tissue using the ADC map and the pre- and post-contrast T_2 and T_2* maps in a multispectral approach [2,3]. *μCT angiography*: Upon sacrifice, mice were perfused with lead chromate latex MICROFIL (Flowtech). *Ex-vivo* tumors were imaged on a SCANCO Medical μCT 40 system (45kV, 177μA, 450ms, 16μm isotropic voxels). The vascular network and tumor volume were automatically extracted from the images [4] and vascular density was calculated as vascular volume/tumor volume. *Experimental details:* VSI MRI was performed pre- and 24h post-tx with 10mg/kg GDC-0980 (n=9) or methylcellulose/Tween-80 (MCT vehicle control, n=9). *Ex-vivo* μCT was performed in a 2nd cohort of mice 24h post-tx with 10mg/kg GDC-0980 (n=10) or MCT (n=10).

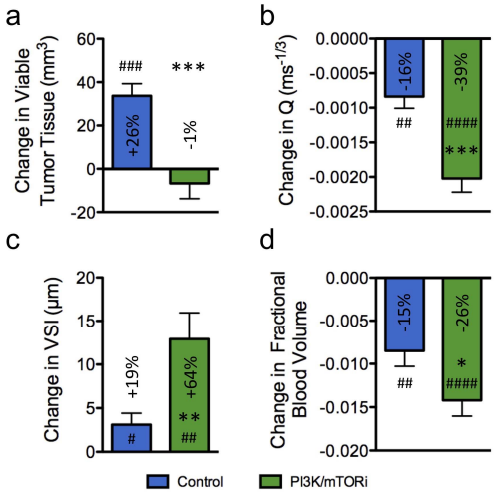

Fig 1. Change in (a) viable tumor tissue, (b) Q, (c) VSI, (d) fractional blood volume 24h post-tx (avg± SEM). * p<0.05, ** p<0.01, *** p<0.0005 t-test vs control. # p<0.05, ## p<0.005, ### p<0.0005, #### p<0.0001 in paired t-test vs pre-tx.

Results. Inhibition of the PI3K and mTOR pathways resulted in significant growth suppression of viable tumor tissue as well as changes in vascular structure as demonstrated by *in-vivo* multispectral VSI MRI (Fig 1,2) and confirmed by the independent *ex-vivo* μCT angiography study (Fig 3). More specifically, vehicle-treated viable tumor tissue grew an average of 34±17mm^3 in 24h (p<0.0005 vs baseline), while PI3K/mTORi-treated viable tumor volume remained static (-7±21mm^3, p<0.0005 vs control, Fig 1a). Structurally, a single dose of the dual PI3K/mTORi reduced the VSI-derived Q by 39% in 24h (-0.0020 ±0.00060ms$^{-1/3}$, p<0.0001 vs baseline, Fig 1b,2), which was significantly reduced relative to the changes observed in the vehicle-treated tumors (-0.00084±0.00051ms$^{-1/3}$, p<0.0005 vs PI3K/mTORi, Fig 1b,2). This result is further corroborated by the *ex-vivo* μCT data demonstrating a -54% difference between control- and PI3K/mTORi-treated vascular density 24h post-treatment (Control: 0.026±0.0063, PI3K/mTORi: 0.057±0.014, p<0.0001, Fig 3). In addition, PI3K/mTORi significantly increased the VSI by 13±9.1μm in 24h (p<0.005 vs baseline and p<0.01 vs control, Fig 1c) and decreased the fractional blood volume by -0.014±0.0054 (p<0.0001 vs baseline and p<0.05 vs control, Fig 1d), consistent with the loss of small vessels.

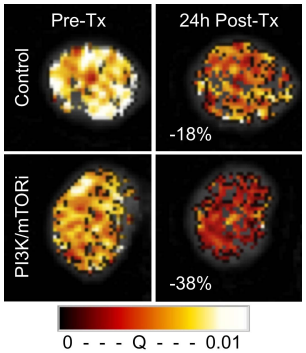

Fig 2. Representative viable tumor Q maps overlaid onto their corresponding M_0 images pre-tx and 24h post-tx with MCT or PI3K/mTORi.

Discussion. Overall, we have demonstrated the ability of multispectral VSI MRI to detect vascular structural changes *in vivo* in response to dual PI3K/mTORi, which are consistent with those observed by *ex-vivo* μCT angiography. Furthermore, these results help elucidate the currently unknown effects of inhibiting the PI3K and mTOR pathways on vascular structure.

[1] Schnell *et al*., Cancer Res. 2008: 6598-6607. [2] Berry *et al*., MRM. 2008: 64-72. [3] Ungersma *et al*., MRM. 2010: 1637-1647. [4] Shojaei F *et al*. Nature 2007; 450: 825-831.

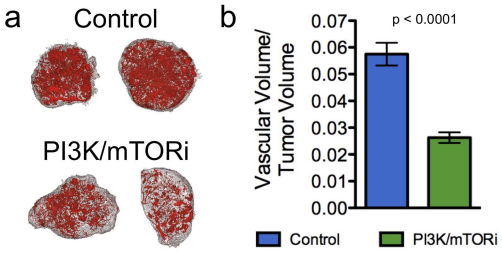

Fig 3. (a) Representative volumetric μCT-angio renderings 24h post-tx with MCT or PI3K/mTORi. (b) Mean vascular density 24h post-treatment with MCT or PI3K/mTORi (avg± SEM).