Noninvasive assessment of functional tumor microvasculature and drug delivery associated with angiotensin receptor blockade in pancreatic cancer

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a devastating illness that responds poorly to chemotherapy. The poor survival of patients with unresectable PDAC may be associated with tumor desmoplasia, which is a significant barrier to drug delivery in pancreatic tumor models. Independent of its potential role in angiogenesis inhibition, Losartan and angiotensin receptor blockade (ARB) has been shown to minimize collagen accumulation and fibrosis in cardiac and renal disease, while demonstrating improvements in perfused vasculature, vessel size, and improved drug delivery in pancreatic cancer mouse models.[1,2] Furthermore, in a cohort of patients with locally advanced or metastatic pancreatic cancer, Nakai et al observed improved progression free survival (PFS) with angiotensin receptor blockade.[3] The goal of this study was to test the hypotheses that MRI with magnetic nanoparticles can provide a non-invasive, steady-state surrogate biomarker of the functional, malignant neovasculature of PDAC in an orthotopic mouse model in addition to providing a surrogate of drug delivery as measured by ^{18}F-5fluorouracil (5FU).

Materials and Methods:

Animal model and tumor treatment protocol: Human pancreatic adenocarcinoma (PDAC) specimens were collected in accordance with IUCAC regulations. Orthotopic tumors were generated by implanting 1mm3 chunks of AK4.4 spontaneously generated tumors (from a Ptfl-Cre/LSL-KrasG12D/p53Lox mouse model) into the pancreas of 6-8 week old FVB mice. AK4.4 pancreatic tumors were separated into two groups (losartan and control), approximately 20 per group, post tumor implantation. Animals were treated daily with an intraperitoneal injection (i.p.) of losartan (70mg/kg) or control (150ul of Normal Saline) for 5 days. ***Magnetic resonance imaging*** was performed at 4.7T on a Bruker imaging system (Biospin, Karlsruhe, Germany). Animals were imaged immediately after five days of therapy. Animals were anesthetized during imaging with 1-1.5% inhaled Isofluorane, and monitored during imaging with respiratory monitoring. Imaging protocols included a Tri-plane and axial RARE localizer. Multi-slice multiecho (MSME) T2-weighted imaging was performed prior to and following intravenous injection of magnetic nanoparticle (MNP)(3.3mg/kg Fe, (Ferumoxytol, Feraheme ® AMAG Pharma. Waltham MA). The following parameters were utilized: Flip angle = 90; Matrix size (128 x 64); TR = 2000msec.; TE = 6 equally spaced echoes at 10msec. intervals; field of view (FOV) = 4.24 x 2.12 cm, slice thickness = 1mm and multiecho gradient echo TE 3.5, 8.5, 13.5, 18.5; TR 750ms). ***MRI data analysis:*** All data was analyzed in Matlab using code written in-house. VVF, VSI and VDI values were obtained by defining a region of interest over the entire tumor area. This process was repeated for 3 central slices of the tumor for every animal, and the mean value within the region of interest (ROI) was calculated. T2 and T2* values were obtained, using GRE and SE data respectively, by plotting mean ROI value at each echo, and calculating the best-fit exponential decay function. R2 and R2* were defined as the inverse of T2 or T2* values; ΔR2 and ΔR2* were calculated as the ratio of values before and after iron contrast injection. VVF of the tumor was derived from the relationship of ΔR2* between muscle and tumors where VVF_{muscle} is assumed to be a constant of 3%. VSI and VDI were calculated using the relationships illustrated below. Paramagnetic maps were generated by calculating VSI, VDI and fBV values on a voxel-by-voxel basis. Histograms were obtained by plotting vessel indices within a tumor region-of-interest against the frequency of occurrence. Data are reported as VVF, VSI and VDI +/- standard error of the mean.[4-7]

$$VVF_{tumor} = VVF_{muscle}\frac{\Delta R^*_{2,tumor}}{\Delta R^*_{2,muscle}} \qquad VSI = 0.424\left(\frac{ADC}{\gamma\Delta\chi B_0}\right)^{1/2}\frac{\Delta R^*_2}{\Delta R_2} \qquad VDI = 329\frac{\Delta R_2}{\Delta R^*_2}$$

Positron Emission Tomography MicroPET studies were performed on a Triumph PET/CT Scanner. N=5 pairs of orthotopic pancreatic tumor model mice were anesthetized using isoflurane and imaged in Treated-Control pairs. Dynamic PET images were acquired for 60 minutes, using a ^{18}F-5FU tracer dose of 200 uCi per animal. ^{18}F-5FU synthesis described elsewhere.[9] CT scans for attenuation and anatomic coregistration were performed immediately following PET acquisition. ROI analysis was performed on dynamic co-registered images using Osirix ® with time activity curves normalized to muscle. Initial area under the curve was performed on normalized curves and then compared to controls. **Statistical Analysis** Statistical analyses compared both cohorts (Losartan (n=19) and Control (n=20)) using an unpaired two-tailed t-test of unequal variances.

Results:

Figure 1 demonstrates pseudocolorized VVF (a,b), VSI (e,f), and VDI (I,j) superimposed over T1-weighted post contrast images at the level of the middle of the orthotopic tumor in the region near the tail of the pancreas. Note the heterogeneous increase in VVF and VSI in the losartan treated cohort as compared to the control, saline treated cohort. This is corroborated by histogram analysis of these data demonstrating a shift in the losartan treated cohort as compared to the control. ROI analysis of the three central slices of the tumor demonstrated a nearly 2 fold increase in VVF and VSI in the losartan treated group as compared to the control group (mean ± sem) (VVF (12 ± 1.7) vs. (6.7 ± 1.2) (p<0.01)) and (VSI (128.2 ± 35.6) vs. (57.5 ± 18.0) (p<0.05)). Paradoxically, there was an increase in VDI (41.7 ± 12.8) vs. (149 ± 56.7) (p<0.05). ^{18}F-5FU microPET was performed in n=5 losartan treated animals with paired controls. Figure (m,n) demonstrates attenuated corrected microPET images in a control animal (m) as compared to losartan treated (n) at 60 min. post injection. Kinetic data was acquired for 80 min. post injection. Area under the curve analysis demonstrate a 53% (Fig. o,p) increase in 5FU delivery within the tumor as compared to the treated animals (p<0.001).

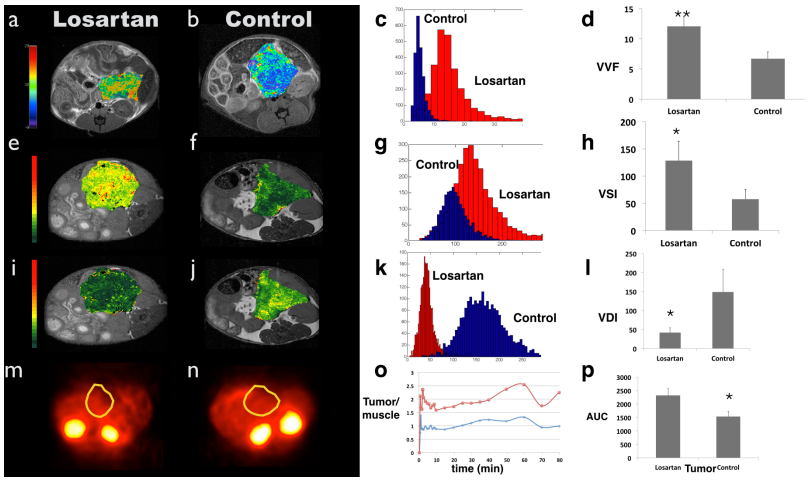

Figure 1: Figure1 represents MRI analyses representing and comparing mice treated with Losartan (70 mg/kg i.p. q.d.) (n=19) for 5 days compared to control mice treated (saline vehicle). Overlying the tumor is a pseudo colorized parametric map of vascular volume fraction (VVF) (a,b), vessel size index (VSI) (e,f), and vessel density index (VDI) (i,j) derived from steady state MRI and administration of magnetic nano particle ferumoxytol (Feraheme (R)) (2.5mg/kg Fe). epresents average (n=5) time activity curves for uptake from an ROI overlying the entirety of the tumor normalized to muscle activity. Figure 2e represents area under the curve analysis of tumor activity normalized to muscle activity in n=5 animals

Discussion:

This data demonstrates that ARB produces a nearly 2 fold increase in VVF and VSI, which is nearly identical to the histologic measures from literature, with a decrease in VDI comparing losartan treated animals to controls. In addition, and again, replicated, noninvasively and in vivo in our AK4.4 mouse model, is a demonstration that losartan demonstrates an approximately 53% in the delivery of ^{18}F-5FU to the tumor as compared to the control group. Recent literature also demonstrated a statistically significant increase in delivery to the tumor of approximately 80%. In summary, we demonstrate a non-invasive technique that is readily translatable to humans, showing strikingly similar quantitative rigor to microscopic techniques as demonstrated in a recent article. Furthermore, this work reinforced that ARB is a robust, reproducible pancreatic cancer model demonstrates increased VVF and VSI, with a discordant decrease in vasculature. These findings support the hypothesis that ARB increases functional microvasculature, and more importantly, that this noninvasive technique is sensitive to these changes.

References

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