Assessment of Radiation Mediated Gene Therapy via Multi-Modality Imaging

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Introduction: Here we report the use of multi-modality, non-invasive imaging for early analysis of spatial patterns of tumor response to combined radiation and antivascular gene therapy. A genetically modified adenoviral vector (Ad.EGR-TNFα), which causes infected cells to express a gene for the production of tumor necrosis factor alpha (TNFα), is upregulated by a radiation inducible promoter, only when those cells have been given a significant dose of radiation. Changes of vascular permeability and perfusion maps using MRI, and tissue oxygenation measurements using electron paramagnetic resonance imaging (EPRI) were registered to characterize a "signature" for response. This "signature" could be used for image guided, fractionated therapy.

Methods: Female athymic nude mice were inoculated with PC-3 xenografts in the right hind limb. On the initial treatment day ("day 0"), both EPRI and MRI imaging were performed, followed by injection of the Ad.EGR-TNF vector, and irradiation with X-ray to 10 Gy, 2-3 hours post injection (N=3). A null vector without radiation was used as a control (N=5). An additional six mice were scanned with DCE-MRI only (5 Ad.EGR-TNF and 1 null). Three days later ("day 3"), the mice were again imaged with EPRI and MRI. No additional doses of either viral vector or radiation were given after day 0. All mice were anesthetized during all procedures. MRI Images were acquired at 4.7 T, using a custom birdcage coil around the tumor bearing leg. T_2 weighted spin echo was used for image registration and anatomical guidance. Dynamic-contrast-enhanced MRI (DCE-MRI) was acquired with a temporal resolution of 6 sec. Uptake of contrast agent (Gadodiamide, 0.015 mM/kg IV) in the dynamic T_1 MR images was fit to an empirical model [1]. EPRI was performed at 250 MHz and 9 mT to acquire a 4D spectral-spatial image. The fitted Lorentzian linewidth, which is broadened linearly with oxygen concentration, was used to map the pO_2 with approximately 1 mm spatial and 3 mm Hg pO_2 resolution. All data are presented as the mean group values ± SEM. A student's t-test was performed to determine significance and a level of $p < 0.05$ was considered significant.

Results: Using a manual image registration method developed in house, we correlated the EPRI-derived oxymetric measurements with the MRI vasculature/blood volume images (Fig. 1). Oxygen increased significantly in all segmented ROIs in all mice receiving TNFα + 10 Gy compared to null vector alone ($p < 0.025$). The rim of the tumor had an increase oxygen of 19 mm Hg relative to day 0 ($p = 0.04$). The tumors in the control mice did not have a significant change in pO_2 after null vector treatment. The contrast uptake rate was lower in the cores of tumors after treatment with TNF + 10 Gy compared to null vector (0.25 ± 0.06 vs. 0.65 ± 0.13 min^{-1}, $p = 0.01$). The null vector treated tumors' cores had an increase in the contrast uptake rate compared to day 0 (0.28 ± 0.08 vs. 0.65 ± 0.13 min^{-1}, $p = 0.03$). The overall contrast agent washout rate was higher in the rims of the TNF + 10 Gy treated tumors when compared to null vector treatment (0.022 ± 0.002vs. 0.012 ± 0.002 min^{-1}, $p = 0.029$). Fig. 1 shows an example of an increase in contrast media uptake rate with a concomitant increase in oxygen. In the figure, the 'initial area under the curve' of contrast media concentration over time is used as a high signal-to-noise ratio measure of contrast media uptake rate – to facilitate pixel-by-pixel analysis. In this extreme case the tumor core appears to be necrotic and then reperfused after treatment.

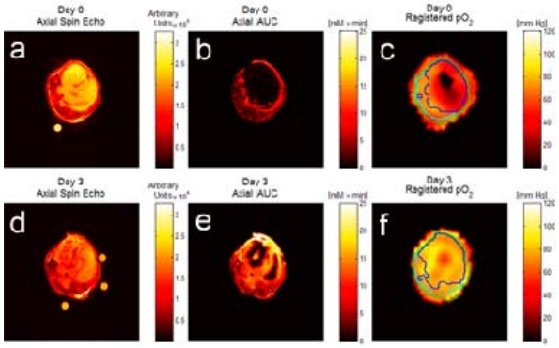

Figure 1 Example of images before (a, b, and c) and after TNF + IR treatment (d, e, and f). Spin echo (T_2) MRIs are shown in (a) and (d). The initial AUC is shown in (b) and (e). Registered EPR oxygen images are shown in (c) and (f) with contours from MRI overlaid. Blue contour denotes the tumor, cyan denotes non-tumor, and green is the outline of the leg.

Conclusions: This is the first report of quantitative, absolute oxygen measurements being correlated with tissue perfusion, in vivo and non-invasively. Image registration facilitated interpretation of the functional images (EPRI, DCE-MRI) aided by the higher resolution anatomical image (T_2 weighted MRI). Radiation mediated antivascular therapy significantly improves tissue oxygenation. The change in oxygenation is associated with a change in the pattern of perfusion; contrast media uptake rate decreases and washout rate increases, suggesting a change in micro-anatomy and physiology. This is consistent with Jain's concept of 'vascular normalization' [2]. The imaging methods described here could be used in an adaptive image guided approach for this new antivascular therapy.

References: 1. Fan, X., et al., Magn Reson Med, 2004. **51**(3): p. 487-94. 2. Jain, R.K., Nat Med, 2001. **7**(9): p. 987-9.