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Characterization of Response to Radiation Mediated Gene Therapy via Multi-Modality Imaging

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Abstract

Imaging techniques are under development to facilitate early analysis of spatial patterns of tumor response to combined radiation and antivascular gene therapy. A genetically modified, replication defective adenoviral vector (Ad.EGR-TNF α), injected intratumorally, mediates infected cells to express tumor necrosis factor alpha (TNF α), which is increased following exposure to radiation. The goal of this study was to characterize an image based “signature” for response to this combined radiation and gene therapy in mice with human prostate xenografts. This study is part of an imaged guided therapy project where such a signature would be useful in guiding subsequent treatments. Changes in the tumor micro-environment were assessed using MRI registered with electron paramagnetic resonance imaging which provides images of tissue oxygenation. Dynamic contrast-enhanced MRI was used to assess tissue perfusion. When compared to null vector (control) treatment, the ratio of contrast agent (Gd-DTPA-BMA) washout rate to uptake rate was lower ($p = 0.001$) after treatment, suggesting a more balanced perfusion. Concomitantly, oxygenation significantly increased in the treated animals and decreased or did not change in the control animals ($p < 0.025$). This is the first report of minimally invasive, quantitative, absolute oxygen measurements correlated with tissue perfusion in vivo.

Keywords

Gene therapy; hypoxia; DCE-MRI; EPRI

Introduction

One potentially important application of functional image information is in the planning, guidance, and assessment of therapeutic interventions. Radiation treatment of cancer has long been an image guided therapeutic modality. Extensive 3D anatomic information from CT and MRI came into widespread use for radiation treatment planning in the 1980's and 1990's. Currently, fused multimodality patient models incorporating both anatomic and functional image information are routinely used. In addition to its obvious utility in identification of active tumor foci not apparent on anatomic images, use of functional image information has been proposed both as a means of identifying important functional regions at risk for damage, in

order to design a treatment which avoids them (1,2) and as a means of identifying subregions within an overall target volume (3). The current generation of radiotherapy treatment devices support spatially nonuniform beam delivery (intensity modulated radiotherapy, IMRT) which permits the generation of deliberately inhomogeneous dose distributions in a “simultaneous integrated boost” (SIB) designed to deliver extra dose where it is needed (4), as well as conformal avoidance of irradiation to critical normal tissues at risk.

One aspect of physiology which may be particularly important in optimizing radiotherapy is the level of oxygenation within tissues containing tumor cells. It is well established that for the X-rays commonly used in therapy, sensitivity to cell killing can vary dramatically with local oxygen tension, with approximately three times more dose required for the same level of cytotoxicity in hypoxic vs euoxic conditions. In vitro, in vivo, and clinical studies have all confirmed the significance of oxygenation in determining radiosensitivity and predicting (or limiting) success of radiotherapy (5,6,7,8). However, currently available techniques, including the use of percutaneously placed electrodes or implanted electron paramagnetic resonance (EPR) probes reporting oxygen tension at a few points, do not facilitate the repeated measurement of tissue oxygenation throughout a tumor volume, as would be preferred for planning therapy and/or monitoring response. Nonetheless, work combining the use of these localized oxygen measurements with flow measurements have provided important insight into radiosensitization (9-11). One promising and considerably less invasive modality for quantitative oxygen measurement is EPR spectroscopic imaging (8,12,13), where a contrast agent (“spin probe”) is administered intravenously, whose spectrum changes in a known manner as a function of local oxygen concentration. Oxygen imaging based on EPRI has been validated using an Oxylite optical oxygen probe as a gold standard (12). Spatial and oxygen resolutions on the order of 1.5 mm and 3 torr respectively are achieved with currently available technology, though application is currently limited to preclinical imaging of small and medium size animals.

Beyond therapy planning, adaptive optimization of fractionated treatments using functional image information is as yet a largely unexplored capability. In concept, adaptive image guidance for fractionated therapies such as radiotherapy could involve the detection of an image-derived “signature” of treatment effect following each fraction, the signature ideally reflecting the spatial distribution of response, or lack thereof, within the tumor. Based on this identification of responding and non-responding regions, the spatial distribution of dose in subsequent fractions could be modified in order to deliver additional dose to less well responding or “undertreated” regions.

This concept is also applicable in combination therapies, where the spatial distribution of any or all of the combined modalities could be adapted based on functional image feedback after each fraction. In the overall project of which the present work is a part, a radiation inducible antivascular gene therapy is combined with radiation. In this scheme, gene expression is spatially and temporally controlled by irradiation. For the modality used here, radio-inducible elements from the Early Growth Response-1 (EGR-1) gene promoter are inserted upstream of a cDNA encoding Tumor Necrosis Factor-alpha (TNF α). Replication-defective adenovirus is used as the vector, and the construct is called Ad.EGR-TNF α . TNF α is a potent antitumor and antivascular agent, with a variety of potential undesirable side effects on normal tissues. By limiting its expression only to the areas irradiated, the systemic side effects may be minimized, while the synergistic effect of irradiation and TNF α is exploited (see (14) for preclinical toxicity). Preclinical studies using histology and immunohistochemistry have shown that tumors infected with Ad.EGR-TNF α respond to radiation with induction of TNF α expression and substantial increases in antitumor activity (14-18), and clinical trials in several anatomic sites have been initiated. Ultimately, this form of therapy is likely to be delivered in a fractionated scheme, with daily doses of radiation and semi-weekly or weekly injections of

gene vector. In an adaptive image guidance scheme, both the radiation dose distribution and the injection pattern of the gene vector could be subject to adaptation based on functional image feedback. It is the premise of the present work that information from EPR imaging concerning distribution of tissue oxygenation, combined with information on vessel status and tissue perfusion from dynamic contrast-enhanced MR imaging (DCE-MRI), can be used to identify the desired signature of tumor response to this combined therapy, and thus provide the basis of an adaptive image guidance scheme. This multimodality approach allows the assessment of changes in at least two important physiologic variables: oxygen distribution, reflecting possible reoxygenation of previously hypoxic regions; and vascular perfusion, reflecting possible changes in vascularity due to the combined antivascular gene therapy and radiation.

It is possible that in characterizing a “signature” of response to therapy, one may observe counter-intuitive responses. For example, a time dependent increase in oxygenation after antiangiogenic/antivascular therapy has indeed been reported (9,19-23). This highlights an apparent paradox of antiangiogenic therapy: how can antiangiogenic therapy, which is aimed at the destruction of blood vessels to starve the tumor, enhances the response to cytotoxic or radiation therapies, which require blood vessels for delivering drugs and oxygen? Jain suggested a “vascular normalization” in response to antiangiogenic therapy, in which the immature blood vessels are pruned leaving the more patent, mature vessels (22,24). Questions have arisen concerning the scheduling of antivascular or antiangiogenic agents, whether before or after radiation therapy, for maximum effect (9,20,25). The optimal scheduling of antiangiogenic agents with cytotoxic agents such that efficient delivery of the drugs is maximized, possibly via pruning of immature tumor vasculature, has been referred to as the “normalization window” (9,20,22,26). In addition, the time course for tracking response to and administering antivascular/antiangiogenic therapy is critical (9,20-23,25,26). The present paper summarizes our experience to date with development of EPRI and MRI techniques and analysis toward this end.

MATERIALS AND METHODS

Animal protocol

Female athymic nude mice (average weight 25 g, approximately 10 weeks old, from Taconic, Hudson, NY) were injected subcutaneously with 1×10^7 PC-3 human prostate cancer tumor cells (American Type Culture Collection, Manassas, VA) in the right hind limb. The mice were imaged and treated in approximately two weeks. On the initial treatment day (“day 0”), both EPRI and MRI imaging were performed, followed by injection of the Ad.EGR-TNF vector (2×10^8 plaque forming units), and irradiation with 10 Gy of 250 kVp X-ray (Philips RT 250, Philips Healthcare, N.A., Bothell, WA) 2-3 hours post injection (N=3). Ad.EGR-TNF vector has two fewer deletions than the vector being used in clinical trials. As a control, mice were injected with a null vector alone (N=5). 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. An additional six mice had only DCE-MRI (5 treated and 1 control).

All mice were anesthetized during imaging with 1.5 to 3% isoflurane mixed with air to maintain a surgical plane of anesthesia. A standard 24 gauge IV catheter was inserted into the tail vein for the injection of contrast agents. Temperature of the mice was maintained at 37 °C during imaging procedures via warm air (MRI) or radiant heat (EPRI). While not entirely noninvasive considering the IV administration of spin probe and use of the transurethral bladder catheter, the EPRI oxygen measurement causes no damage to tissue in the imaged region and is well suited to repeated studies, in contrast with needle-based percutaneous measurement techniques using electrodes or optical probes. All animal procedures were performed under Institutional Animal Care and Use Committee approved protocols.

MRI

Images were acquired at 4.7 T, using a 30 cm diameter bore GE Omega/ Bruker MRI Scanner equipped with self-shielded imaging gradients with a custom 8-leg low-pass, volume birdcage coil around the tumor bearing leg. For image registration and anatomical guidance, the multi-slice, Rapid Acquisition with Relaxation Enhancement (RARE) spin-echo sequence (TR = 3000 ms, effective TE = 56 ms, FOV = 3.0 cm, matrix size = 256×256 , slice thickness = 0.6 mm, NEX = 2, RARE factor = 8) was used. Images were acquired in planes roughly orthogonal to the long axis of the leg, approximating a transaxial plane. The tumor was identified by a user defined threshold. Tumor volume was measured by semi-automatically drawing an ROI of the tumor for each slice in these T₂ weighted MRI images. The tumor areas for each slice were multiplied by the slice thickness and summed to calculate the tumor volume.

For perfusion imaging, T₁ weighted images were acquired using a Fast Low Angle Shot (FLASH) gradient-echo sequence (TR/TE = 30 ms/3.25 ms, flip angle = 20°, FOV = 2.25 cm, matrix size = 128×128 , slice thickness = 0.6 mm, NEX = 1), typically with 3 slices. 128 evolution cycles were taken without a delay between scans with a temporal resolution of 3.8 sec followed by another 128 evolution cycles with 5 sec delay between scans to capture more of the washout phase. Uptake of contrast agent (Omniscan® (Gadodiamide, Gd-DTPA-BMA), 0.15 mM/kg IV, GE Healthcare, Piscataway, NJ) in the dynamic T₁ MR images was analyzed to assess vascular structure and function. Total imaging time for MRI was typically 1.25 hr.

Kinetic modeling—Using the T₂ weighted image as a template, ROIs were drawn for the tumor and muscle semi-automatically with a user defined threshold. The tumor ROI was further subdivided into rim and core ROIs. A muscle-tumor boundary ROI was defined as three voxels from the tumor ROI into the muscle. To minimize the effect of tumor cells or abnormal blood vessels at the boundary of the tumor, this muscle-tumor boundary ROI was excluded from the muscle ROI. For each non-muscle ROI, pixels were clustered based on a histogram of the initial area under the contrast uptake curve (iAUC) using connected components labeling (bwlabel, part of the MATLAB Image Processing Toolbox, The MathWorks, Natick, MA). This process “labeled” each cluster, defined as four or more spatially connected pixels, within the low, medium and high uptake thirds of the iAUC histogram. To increase signal-to-noise-ratio, the pixels within each cluster were averaged for kinetic modeling. For the muscle ROI, only a single cluster was used.

The early increase in image intensity due to the contrast agent (CA) was used to calculate tissue concentration of the CA. A small ROI of the muscle was used to convert the DCE-MRI signal into gadodiamide concentration according to the relation

$$C(t) = \frac{1}{R_1 \cdot T_{1(\text{muscle})}} \cdot \frac{S(t) - S(t_0)}{S_{\text{muscle}}(t_0)}$$

Where C(t) is CA concentration as a function of time, R₁ is relaxivity of gadodiamide in plasma at 37 °C and 4.7 T, T₁ is relaxation time of a reference tissue, and S is signal.

An empirical mathematical model (EMM) was used to characterize the CA kinetics (27,28).

$$C(t) = A \cdot (1 - e^{-\alpha t})^q \cdot e^{-\beta t} \cdot \frac{1 + e^{-\gamma t}}{2}$$

Where A is the upper limit of contrast concentration, α [min⁻¹] is the rate of contrast uptake, β [min⁻¹] is the overall rate of contrast washout, γ [min⁻¹] is the initial rate of contrast washout,

and q is related to the radius of curvature of $C(t)$ at the transition from first-pass uptake to initial washout.

The classic two-compartment model did not adequately describe the tracer kinetics of these PC-3 tumors and therefore was not used in our analysis. Others have also found that if one was interested in the washout period, i.e., the kinetics beyond about 10 minutes, the two-compartment model (TCM) fit was very poor (29,30).

A nonlinear least-squares optimization routine (lsqcurvefit, part of the MATLAB Optimization Toolbox) was used with physiologically determined parameter limits for fitting pharmacokinetic models to the DCE-MRI data.

EPRI

The EPR imaging methodology has been described earlier (31,32). Briefly, the spin probe used for EPRI was a carbon centered trityl radical, OX063 (2 g/kg, GE Healthcare, London, UK), injected as an IV bolus. Unless preventive measures are taken, the EPR contrast agent accumulates in the bladder, creating large image artifacts due to the proximity of bladder and leg-borne tumor. To address this problem, we developed a double lumen catheter which was inserted into the bladder via the urethra, and was flushed continuously during EPR imaging with water (33). A 1.6 cm diameter loop-gap resonator was used for EPRI at 9 mT (250 MHz) using a locally developed spectrometer. A 4D spectral spatial image was acquired with 10×10 spatial and 14 spectral projections with FOV = 3.96 cm, and reconstructed using filtered back projection to yield a 64 point spectrum at each voxel. The reconstructed voxel spacing was 0.62 mm. The spectrum at each voxel was fitted to a physics-based model (34). The fitted Lorentzian linewidth, which varies linearly with oxygen concentration, was used to compute the pO_2 at each voxel. The linewidth vs pO_2 characteristic of OX063 was calibrated utilizing samples equilibrated with gas mixtures of known oxygen concentration.

Each of the EPR oxygen images was performed within 2 hours before the corresponding spin-echo and dynamic MRI scans. Thus, we have blood vessel and oxygen information on the same tumors at essentially corresponding times on each day pre- or post-treatment. It is therefore assumed that differences in metabolic state can be ignored. Total imaging time for EPRI was typically 1 hr.

Image registration

Using a locally developed manual image registration method, we correlated the EPRI-derived oxymetric measurements with the DCE-MRI. The tumor bearing legs were immobilized for imaging using a vinyl polysiloxane dental material cast (GC America). It has been shown previously that the dental material does not affect image quality (35).

Statistical analysis

All data are presented as mean value $\pm$ SEM. An independent student's t -test was performed to determine significance between treatment groups and a paired student's t -test was performed to determine significance of differences before and after treatment. A paired samples correlation table was also used to determine if a change in a value was correlated with treatment. Levene's Test for equality of variances was performed to test for heterogeneity of variance. All analyses were performed using SPSS v15.0 (SPSS Inc, Chicago, IL) and a level of $p < 0.05$ was considered significant.

RESULTS

Baseline tumor volume was not significantly different ($p = 0.6$) between the TNF α + 10 Gy group ($288 \pm 74 \mu\text{L}$) and null vector alone group ($231 \pm 75 \mu\text{L}$). Three days after treatment, the mean tumor volume of the TNF α + IR group was $370 \pm 87 \mu\text{L}$. The mean tumor volume of the null vector alone group was not significantly different from either pre-treatment or the TNF α + IR group at three days after treatment ($421 \pm 97 \mu\text{L}$, $p = 0.2$ and $p = 0.7$, respectively). Note that a single fraction of TNF α + IR treatment is not curative, so no significant tumor control at three days was expected or observed.

Oxygen

MRI-defined anatomical ROIs were mapped onto the EPRI oxygen images using our image registration software. Oxygen increased significantly in all segmented ROIs in all mice receiving TNF α + 10 Gy compared to null vector alone ($p < 0.025$). The rim of the tumor had an increased $p\text{O}_2$ of 19 mm Hg relative to day 0 ($p = 0.04$). The tumors in the control mice did not have a significant change in $p\text{O}_2$ after null vector treatment (Fig.1).

DCE-MRI

We describe the results for parameters of the empirical mathematical model only, since as discussed earlier the two compartment model does not adequately describe the CA kinetics in the PC-3 tumors and 30 min DCE-MRI acquisition used here. The use of a low molecular weight CA (Gd-DTPA-BMA) implies that perfusion is more dominant than permeability.

The CA uptake rate (α) typically increased three days after null vector treatment alone, while three days after TNF α + IR treatment the CA uptake rate was essentially unchanged (Fig. 2). Specifically, three days after null vector treatment alone, the α value of the tumor ($0.91 \pm 0.21 \text{ min}^{-1}$) was higher compared to day 0 ($0.40 \pm 0.07 \text{ min}^{-1}$, $p = 0.09$) and also compared to three days post TNF α + IR treatment ($0.43 \pm 0.07 \text{ min}^{-1}$, $p = 0.02$). In particular, the core of the tumor had a greater α value three days after null vector treatment alone ($1.03 \pm 0.11 \text{ min}^{-1}$), compared to pre-treatment ($0.36 \pm 0.10 \text{ min}^{-1}$, $p = 0.01$) and also compared to three days post TNF α + IR treatment ($0.42 \pm 0.07 \text{ min}^{-1}$, $p = 0.001$).

The rim of the tumors had a significant increase in overall CA washout rate (β) three days after TNF α + IR compared to treatment with null vector alone ($0.030 \pm 0.002 \text{ min}^{-1}$ and $0.018 \pm 0.004 \text{ min}^{-1}$, respectively, $p = 0.004$) and compared to baseline ($0.020 \pm 0.005 \text{ min}^{-1}$ $p = 0.05$) (see Fig. 3).

Three days after null vector alone treatment, the cores of control tumors had an increase in the initial CA washout rate (γ) from $0.53 \pm 0.08 \text{ min}^{-1}$ to $0.83 \pm 0.04 \text{ min}^{-1}$, $p = 0.1$ which was significantly different from TNF α + IR treated mice (0.50 ± 0.06 , $p = 0.01$). This would likely account for the significant difference between the whole tumor ROI initial CA washout rates three days after null vector alone compared to three days after TNF α + IR (0.72 ± 0.06 vs. 0.51 ± 0.05 , $p = 0.03$).

The rim of the tumor, in the null vector alone group had a large increase in CA uptake after treatment without an improvement in oxygenation, while the TNF α + IR group had a significant increase in oxygenation without a significant increase in CA uptake. The control group had an increase in CA uptake (α) and a slight decrease in CA washout (β). The TNF α + IR group had the opposite CA kinetics, i.e., essentially no change in CA uptake with a large increase in CA washout. Actually the ratio of CA washout to uptake was significantly lower when comparing the whole tumor, rim of the tumor and core of the tumor, three days after null vector alone with TNF α + IR ($p < 0.023$). This suggests that in the control group the tumor has an increase in delivery of blood yet oxygenation does not improve, possibly due to higher oxygen

consumption. There is a mismatch between uptake in the rim of the tumor and washout of the core of the tumor. However, in the TNF α + IR group the oxygenation improves, suggesting that the balance between delivery at the rim and core of the tumors improves oxygenation.

Figure 4 and 5 illustrate the information available from a completed experiment using the protocol described here, for a null vector treated and TNF α + 10 Gy treated mouse, respectively. The growth of the tumor can be appreciated in the MRI images (Fig. 4 a, b, d, and e). The water fiducials used to register the MRI with EPRI are visible in the spin echo images. iAUC is shown because of its high signal-to-noise-ratio measure of contrast media uptake rate, to better visualize changes, on a pixel-by-pixel analysis. iAUC of the tumor increased by 23% three days after null vector treatment (Fig. 4 b and e). Looking at the tumor ROI (blue contour) overlaid on the oxygen image, the tumor appears to be more hypoxic three days after treatment with null vector alone. Figure 5 shows an example of an increase in contrast media uptake rate (iAUC increased 449%) in the tumor with a concomitant 58% increase in oxygenation following treatment with TNF α + 10 Gy. In this extreme case the tumor core appears to be necrotic prior to treatment and then partially reperfused after treatment.

DISCUSSION

As early as 1965, thalidomide (an antiangiogenic drug) was used to treat cancer patients (36). Since then, many antivascular or antiangiogenic agents have been used both in clinical and pre-clinical studies (37). There are over 500 antivascular/antiangiogenic cancer related clinical trials ongoing in the United States and Europe (38). Seven of those clinical trials are for TNFerade™, the commercial name for the radio-gene therapy agent, similar to that used in this study. The ability to observe and understand changes to the tumor microenvironment caused by these drugs is indispensable, especially if done non-invasively. For image guided therapy, it is essential that one has an imaging based endpoint that is well characterized. Using MRI and EPRI, we found that TNF α + 10 Gy improves oxygenation with a more balance delivery of blood (as evidenced by an decrease in CA uptake rate with an increase in washout).

Oxygen

We observed increasing oxygenation in the rim of the tumor after antivascular treatment with TNF α plus 10 Gy radiation. It should be noted that the increase in oxygenation of the muscle and muscle-tumor boundary could be attributable to partial volume effects, as the voxel size that was used to define the ROIs was much smaller than the EPRI resolution. The fact that as the tumor grows, the muscle ROI becomes smaller can exacerbate this effect. Also, the DCE-MRI had high in-plane resolution relative to EPRI, but the slice thickness for DCE-MRI was comparable to EPRI resolution. Therefore, DCE-MRI could have similar partial volume artifacts as EPRI, since a mouse blood vessel can be much smaller than the voxel dimension. The DCE-MRI parameters were chosen to balance spatial and temporal resolutions.

In studies using thalidomide and radiation, a window of treatment of two to three days was found, i.e., hypoxia was attenuated up to three days after treatment (9,23). In another study, Hou et al used a hemoglobin allosteric effector to increase oxygen availability within the tumor, to enhance radiotherapy (21). Tumor oxygenation improved after three days of treatment when compared to baseline and was significant after five days of treatment. Using an Eppendorf oxymetric electrode, Dings et al reported an increase in oxygenation up to five days after treatment with bevacizumab, another antiangiogenic agent (20). Although the tumors and methodology were different in these studies, they are consistent with the increase in oxygen three days after treatment reported here.

DCE-MRI

There were cases where the tumor had grown to such an extent that the muscle ROI was difficult to define. Recalling that the muscle is used as a reference to convert the DCE-MRI signal into CA concentration, this can lead to additional uncertainty in the DCE-MRI analysis.

Using an antivascular therapy, we observed in this study that oxygenation of the tumor improves with a concomitant improvement in perfusion of the tumor, i.e., the ratio of CA washout (β) to uptake (α) was more balanced. The improvement in oxygenation and tumor perfusion was achieved by balancing the perfusion of the core and rim of the tumor. A better balance between contrast media uptake and washout suggests less microscopic heterogeneity and therefore be less hypoxic locally. Contrast agent molecules are likely not leaking out in highly vascularized regions and diffusing into very sparsely vascularized regions a couple hundred microns away (this would produce rapid uptake combined with slow washout). Instead the extravascular space is a well mixed compartment so that uptake and washout is balanced, i.e. fast uptake and fast washout.

This more balanced perfusion combined with improved oxygenation around two days after antivascular therapy is consistent among other possibilities, with the concept of “window of normalization”. Jain suggests that suboptimal scheduling of antiangiogenic therapy could lead to antagonism between antiangiogenic and cytotoxic agents and that advances in imaging technologies could identify a “normalization window of opportunity” (22,24). Using DCE-MRI and histology, Ansiaux et al, suggested that after thalidomide treatment, there was a shift to larger blood vessels to compensate for the loss of smaller (immature) vessels (9). As mentioned earlier, they also reported an increase in oxygenation during the same time course. On the contrary, using an oximetric electrode, Bruberg et al found that A-07 melanoma xenografts had no significant change in mean pO_2 one and three days post irradiation with a single fraction of 10 Gy (19). In the same study they reported a decrease in perfusion after a single fraction of 10 Gy. They clearly stated the limitations of the invasiveness of the electrode measurement and the inability to compare it spatially to the DCE-MRI obtained. Therefore, they concluded that it is not possible to determine if the increase in extracellular volume fraction which leads to increased pO_2 (via reduced oxygen consumption) was greater than the reduced oxygen supply (lower perfusion). The use of repeatable, quantitative oxygen measurement combined with registered DCE-MRI ROIs as reported here affords the possibility to differentiate between the causes for a change in oxygenation. After technological improvements to reduce imaging time and increase spatial resolution, registered EPRI and DCE-MRI could be used to compare voxels and determine oxygen consumption and tissue perfusion, serially.

However, one limitation would remain in our method, namely the ability to compare our DCE-MRI results with that of others. As mentioned previously, the TCM has difficulty in accurately characterizing data collected for more than ~ 10 min. However, it is widely used and one would like to not only have a more physiological understanding of the pharmacokinetic models, but to also compare their results. Two major difficulties in comparing the EMM with the TCM is the number of parameters (the TCM has two parameters K^{trans} and v_e , but the EMM has five parameters (A , α , β , γ and q)) and the reliance on an arterial input function for the TCM.

It is possible that TNF α +IR is causing selective vascular destruction. Because of the size of the TNF α protein, 17 kD for the monomer and 51 kD for the active trimer, TNF α diffuses slowly from the point of its synthesis. To locally eliminate the protein, an intact, functioning vascular system is necessary. Areas with chaotic, non functioning vasculature (typical of tumor neovasculature) will then develop much higher concentrations of TNF α and these vessels will more likely be selectively eliminated. In fact, histopathology of muscle tissues has previously shown high levels of TNF after Ad.Egr-TNF α + 20 Gy without vascular thrombosis (18).

Not all reports support the idea of vascular normalization or an increase in oxygenation following antiangiogenic therapy. For example, Fenton et al, showed an increase in hypoxia from a combination of 5×2 Gy/week and an antiangiogenic drug using histological methods (25). In that report, the antiangiogenic agent was given after radiation, unlike the protocol used here.

The use of a single fraction may have limited a potential vascular normalization response, i.e., an increase in CA uptake and washout. However, an increase in oxygenation was seen in the muscle (comparing treated with controls) with only a single fraction ($p = 0.006$), which could also be due to partial volume effects. The disparity in resolution between MRI and EPRI could cause ROIs defined with MRI to include more than one tissue type as the voxel sizes are about three times larger for EPRI.

Another consequence of using just a single fraction is that it was difficult to control the tumor size and/or avoid mouse mortality during the repeated multimodality imaging protocol. The difficulty of multimodality serial imaging should be appreciated. Even if the mouse can survive to day 3 and the tumor is not too large, the tail vein and bladder must be catheterized again. Therefore the number of animals completing the protocol used in the current study was limited. With more animals it should be possible to differentiate between tumors that respond to $\text{TNF}\alpha + \text{IR}$ via direct apoptosis vs the antivasular effect. The difficulties of this protocol also led us to focus on comparing $\text{TNF}\alpha + \text{IR}$ and null vector alone. It is known that irradiation alone changes tumor perfusion and oxygenation such that a null vector + IR group (essentially IR alone) would be desirable. However, previous work using histology has shown that Ad.EGR-TNF $\alpha + \text{IR}$ had a higher cure rate when compared to null vector alone, null vector + radiation, and Ad.EGR-TNF α alone (as some TNF is produced in the absence of radiation) (15,16,18).

In conclusion, this is the first report of quantitative, absolute oxygen measurements being correlated with tissue perfusion, in vivo and with minimally invasiveness. Image registration facilitated interpretation of the functional images (EPRI, DCE-MRI) aided by higher resolution anatomical images (T_2 weighted MRI). Radiation mediated antivasular therapy was observed to significantly improve 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. The data is consistent with the vascular normalization concept as proposed by Jain, which could be due to selective pruning governed by diffusion of the adenoviral vector or the TNF α produced. The imaging methods described here could be used in an adaptive image guided approach for this new antivasular therapy, identifying regions of good and poor response to allow optimized targeting of subsequent fractions.

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Figure 1. Oxygenation before and after treatment. * denotes significantly different from baseline. # denotes significantly different from control. Null vector alone, N = 5; TNF α + IR, N = 3.

Figure 2. α : rate of contrast agent uptake derived from EMM. * denotes significantly different from baseline. # denotes significantly different from control. Null vector alone, N = 5; TNF α + IR, N = 9.

Figure 3.

β : overall rate of contrast agent washout derived from EMM. # denotes significantly different from control. Null vector alone, N = 5; TNF α + IR, N = 10.

Figure 4. Example of images before (a, b, and c) and after null vector alone treatment (d, e, and f). Spin echo 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. Serial images are not registered as there are only 3 slices. However, images are approximately in the same location.

Figure 5.

Example of images before (a, b, and c) and after $\text{TNF}\alpha + \text{IR}$ treatment (d, e, and f). Spin echo 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. Serial images are not registered as there are only 3 slices. However, images are approximately in the same location.