Dynamic-Contrast-Enhanced-MRI shows radiation resistant tumor (Nu61) is also resistant to TNFalpha treatment – pilot study

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Introduction: Vascular permeability and perfusion maps using dynamic-contrast-enhanced MRI (DCE-MRI) were performed to characterize the response of a radioresistant cell line to therapy. Ionizing radiation (IR) is a staple for the treatment of malignant tumors. However, failure to cure tumors is thought to be due to an intrinsic tumor cell radioresistance and the tumor's microenvironment. Nu61 is a radioresistant tumor derived from a human head and neck squamous cell carcinoma (SCC-61) parental line. A genetically modified adenoviral vector (Ad.EGR-TNFα) which causes infected cells to produce tumor necrosis factor alpha (TNFα), is upregulated by a radiation inducible promoter, only when those cells have been irradiated. TNFα 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 TNFα is exploited. In this pilot study, different treatments were evaluated and characterized with DCE-MRI.

Methods: Female athymic nude mice with Nu61 or SCC-61 xenografts in the right hind limb were randomized into one of the following groups: I) Ad.null (Nu61=6, SCC-61=3), II) Ad.null + 10 Gy (Nu61=3, SCC-61=4), III) Ad.Egr-TNF (Nu61=3, SCC-61=4) or IV) Ad.Egr-TNF + 10 Gy (Nu61=4, SCC-61=4), where Ad.null is a control vector. On the initial treatment day, MRI was performed, followed by injection of either vector plus 10 Gy of irradiation, 2-3 hours post injection (depending on the group). Three days later, the mice were again imaged. No additional treatment was given after day 0. All mice were anesthetized during all procedures. Images were acquired at 9.4 T, using a Bruker MRI Scanner with a custom 10-leg low-pass, volume birdcage coil around the tumor bearing leg. For anatomical guidance, the multi-slice, Rapid Acquisition with Relaxation Enhancement (RARE) spin-echo sequence (TR = 4000 ms, effective TE = 28 ms, FOV = 2.56 cm, matrix size = 256 × 256, slice thickness = 0.75 mm, NEX = 2, RARE factor = 4) was used. For DCE-MRI, T_1 weighted images were acquired using a Fast Low Angle Shot (FLASH) gradient-echo sequence (TR/TE = 40 ms/3.6 ms, flip angle = 20˚, FOV = 2.75 cm, matrix size = 128 × 128, slice thickness = 1.5 mm, NEX = 1), with temporal resolution of 5 sec. Contrast agent (CA) (OmniScan) kinetics were fit to a two-compartment model, where K^{trans} is a measure of the rate of uptake of CA and v_e is the volume accessible to the CA. An ANOVA was performed to determine significance and a level of $p < 0.05$ was considered significant. All data are presented as mean ± SEM.

Results: K^{trans} and v_e (in all regions of interest) were not significantly different among the four treatment groups with Nu61 ($p>0.5$). The large difference in K^{trans} with Nu61 cell line is likely due to an outlier in the Ad.Egr-TNF + 10 Gy treated group. However, the tumor region of the mice with the SCC-61 xenografts had a significantly lower K^{trans} 3 days after Ad.Egr-TNF + 10 Gy, when compared to Ad.null or Ad.null + 10 Gy (45% and 50%, respectively with $p=0.03$). In the tumor region, v_e was 64% lower 3 days after treatment when comparing Ad.null alone with Ad.Egr-TNF + 10 Gy ($p=0.006$). The results are summarized in Table 1 for the tumor region, 3 days after therapy.

Table 1: DCE-MRI Results, 3 Days Post Treatment

	K^{trans} [min^{-1}]		v_e	
	Nu61	SCC-61	Nu61	SCC-61
I) Ad.CMV.null	0.03±0.01	0.55±0.09	0.52±0.02	0.67±0.04
II) Ad.CMV.null + 10 Gy	0.05±0.02	0.49±0.07	0.40±0.08	0.53±0.03
III) Ad.Egr-TNF	0.05±0.01	0.22±0.05	0.51±0.06	0.48±0.04
IV) Ad.Egr-TNF + 10 Gy	0.25±0.22	0.25±0.03	0.47±0.07	0.43±0.05

Conclusion: Kinetic modeling of DCE-MRI data suggest that Nu61 tumors, which are radioresistant, are also resistant to TNFα therapy. However the parent cell line, SCC-61 responds to TNFα and irradiation via an apparent decrease in permeability and perfusion. More experiments are in progress to support these promising preliminary results, e.g. positron emission tomography is also being used to characterize these two cell lines. The radiation-induced gene therapy used here is currently undergoing clinical trials. DCE-MRI could be used to guide subsequent fractions in an adaptive image guided approach, possibly identifying non-responding regions.